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WATER SOAKING OF LEAVES IN RELATION TO DEVELOPMENT OF THE WILDFIRE DISEASE OF TOBACCO [1]

By E. E. CLAYTON

Plant pathologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Leaf spot diseases, popularly referred to as red rust, black rust, and white rust, have long been a problem in the culture of tobacco (*Nicotiana tabacum* L.), but until recently nothing was known as to either their cause or prevention. During the period 1917 to 1925, however, serious outbreaks of these leaf-spot diseases led to scientific investigations, and in consequence the two most important types of leaf spot were described respectively as wildfire (*Bacterium tabacum*)[2] by Wolf and Foster (*16*)[3] and blackfire or angular leaf spot (*Bact. angulatum*) by Fromme and Murray (*9*). Seed treatment, seedbed sanitation, and, in some localities, seedbed spraying were recommended as control measures. These proved very effective in eliminating or greatly reducing the amount of seedbed infection, but they have not been effective in preventing destructive field outbreaks of leaf spot.

The field epidemics occur late in the season and have been closely associated with certain weather conditions and fertilization and cultural practices. Recently there has been a growing tendency to question whether either *Bacterium tabacum* or *Bact. angulatum* is capable of causing destructive leaf spot epidemics in the field, and to regard this leaf break-down as, at least in part, a nonparasitic trouble due to unbalanced nutritional conditions, possibly assisted by over maturity of the leaf. In support of this view Valleau (*14*) has cited the fact that *Bact. angulatum* when inoculated into leaves produces only very small lesions of a harmless nature, while field blackfire is characterized by large rapidly developing lesions that cause great destruction. In the earlier work, the fact that the organism could be isolated from the large field lesions and would produce small lesions from controlled inoculations led to the assumption that it would also be able to produce the large type of lesion if suitable conditions were provided.

Exactly the same situation exists with respect to *Bacterium tabacum* and wildfire. Artificial inoculations with the organism have always produced lesions consisting of a small central dead area surrounded

[1] Received for publication Sept. 10, 1935; issued March 1936.
[2] Synonym, *Phytomonas tabaca* (Wolf and Foster) Bergey et al.
[3] Reference is made by number (italic) to Literature Cited, p. 268.

by a broad yellow halo (fig. 1, *A*), and these lesions require about a week to develop fully. In the field, on the other hand, lesions of large size may appear in 2 or 3 days with little or no suggestion of a halo (fig. 1, *B*). The halo-type lesions produced by artificial inocula-

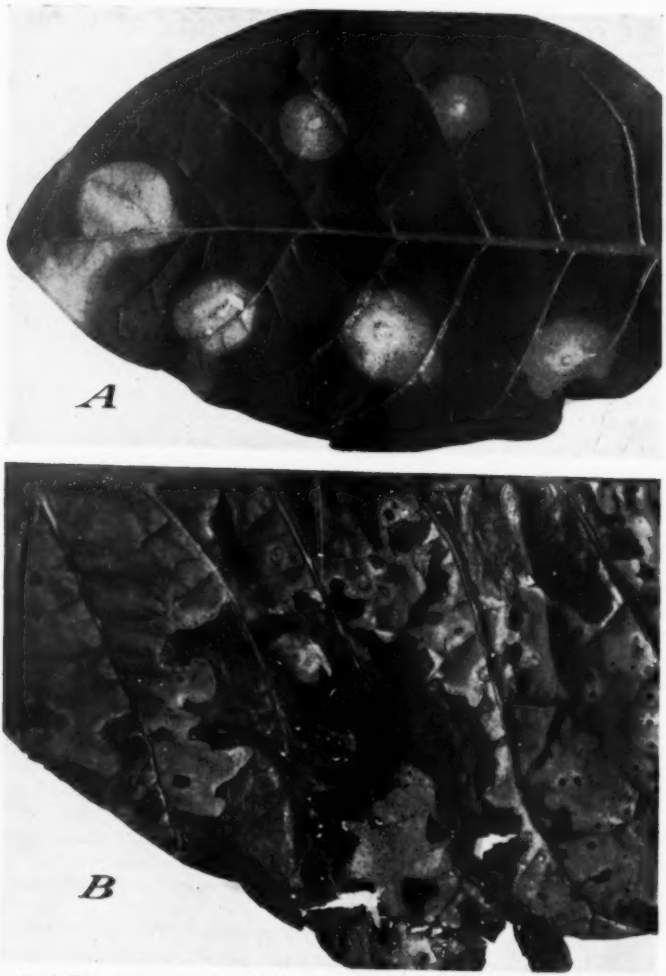

FIGURE 1.—*A*, Halo wildfire. Lesions of this type result when young leaves are pricked with a needle dipped in a *Bacterium tabacum* culture. Note the small central dead area surrounded by a broad yellow halo. *B*, Epidemic wildfire. These lesions are typical of those which developed in the field during the leaf spot epidemic of 1933. Note the large irregular dead areas with little or no evidence of halos around the margins.

tion have been regarded as typical wildfire, but the exact cause of the large field-type lesions, referred to in this paper as "epidemic wildfire", has been questioned by various writers. Thus as early as 1921 Chap-

man and Anderson (*4*) reported that in the Connecticut Valley they found much "rust", which they suggested was probably caused by excess of nitrogen and deficiency of potash. Reference to a nonparasitic rust which might be confused with wildfire is also made by Johnson and Fracker (*11*). Beach (*1*) has logically connected the nonparasitic blackfire suggested by Valleau (*14*) with the nonparasitic rust mentioned above, and he states:

> Nonparasitic spots, including what the farmers have called "rust" but referred to by pathologists as "blackfire", often develop on the tobacco at maturity when the weather is very wet.

Thus, both in the southern tobacco region with *Bacterium angulatum* and in the northern region with *Bact. tabacum*, the leaf spot situation is complicated by the possibility that the destructive late-season epidemics are in part nonparasitic. This view is supported indirectly by the failure of recommended control measures to give protection under field conditions, and directly by the fact that artificial inoculations with either organism produce small harmless lesions (fig. 1, *A*) which fail to develop into anything resembling the large field lesions (fig. 1, *B*). No one, however, has been able to reproduce the large lesions under controlled conditions by either pathological or physiological methods.

In seeking an explanation of this situation, the writer has conducted work simultaneously with the bacteria mentioned and also with various fungi. The present paper, however, records only the results secured with *Bacterium tabacum*.

The fundamental importance of this study lies in the fact that if the destructive, late-season type of leaf spot is not caused by an organism, then obviously the present control methods, which are directed at eliminating the organism, have no chance of success, and the entire problem of leaf spot control must be reconsidered. On the other hand, if the organism can be proved to be an essential factor in leaf spot development, then the failure of the present control program can be safely ascribed to inadequacy of measures recommended and not to misdirection of efforts. Obviously, the only way to prove definitely either the parasitic or the nonparasitic theory of the cause of leaf spot is actually to reproduce the large destructive type of field lesions under controlled conditions.

METHODS OF STUDY

The organism was isolated from material obtained in many localities and from lesions of widely varying appearance. Cultural and inoculation studies with these isolations showed no differences. The pathogenicity of all isolations decreased rapidly in artificial culture, but was readily maintained by frequent inoculation and reisolation. Inoculations were usually made with 6- to 10-day-old broth cultures diluted 10 times with water. Wound inoculations were made with sharp-pointed glass tubes, leaving a small drop of the inoculum at each prick. Following inoculation the plants were incubated in a saturated atmosphere at a favorable temperature (70°–80° F.) for 48 to 72 hours. In prior studies the general practice had been to use very young plants, but in this work large plants were employed for both greenhouse and field studies. Since the different leaves of large plants do not respond in the same manner to infection, it was found advisable to classify

leaves as basal, middle, or tip. All measurements of lesion size refer only to the area of tissue actually dead. Nutrition studies in the greenhouse were conducted with field soils of low fertility to which the various nutrient salts were added in solution at weekly intervals.

Field studies were conducted at the Arlington Experiment Farm, near Rosslyn, Va., and at Upper Marlboro, Md. At the former location the soil was a medium sandy loam of moderate fertility, and at the latter location the soil was a light sandy loam of low fertility. At Upper Marlboro there was available an extensive series of agronomic experiments which provided a wide range of nutritional and topping conditions. The disease was well distributed each year in these plantings, and the epidemic type of wildfire was general in 1933. The Upper Marlboro plantings provided an exceptional opportunity to study the influence of cultural and nutritional factors on disease development. The object of the work necessitated taking data in much detail, often as counts of the number of lesions and measurements of the size of lesions, leaf by leaf. Presentation of any considerable amount of these data is impracticable, and consequently in this report significant developments are summarized and supplemented with representative data. The large volume of negative results secured during the early phases of the study will first be referred to very briefly.

The Maryland Broadleaf variety was used throughout this work, and supplementary tests were conducted with Connecticut Havana and Cash, the latter being a strain of Orinoco.

RESULTS OF PRELIMINARY EXPERIMENTS

It has been suggested that the epidemic type of wildfire is due (1) to intensive dissemination of the organism by rain spattering plus high humidity, and (2) to excessive plant susceptibility induced by low topping and high-nitrogen or low-potash fertilization. During the first year and a half of this study, numerous experiments were conducted along these lines.

Plants in the field were sprayed with bacteria during the progress of rains to find whether intensive inoculation under supposedly favorable natural conditions would be effective. In the greenhouse, plants were similarly inoculated and incubated in a saturated atmosphere for various periods and at different temperatures. Excellent infection, as judged by the number of lesions, was obtained in both field and greenhouse experiments. This was favored by temperatures between 70° and 80° F. and by incubation periods of 48 to 72 hours. The lesions that resulted, however, were all of the halo type, and consequently they bore no resemblance to epidemic wildfire.

In other experiments, the effect of height of topping was studied, and it was found that low topping increased both the number and the size of lesions, but again all lesions were of the halo type. Many infection experiments were conducted with leaves of different ages, with inoculations of both the upper and the lower leaf surfaces, both with and without wounding. Wound inoculations were positive under all circumstances. Without wounding, young leaves and lower surfaces were more easily infected than were old leaves and upper surfaces. The lesions secured were all of the halo type, and the results

tended to indicate that halo and epidemic wildfire are different, for in the field old leaves, and not young ones, were most severely injured.

Extensive nutrition experiments were conducted with nitrogen, phosphorus, potassium, magnesium, and chlorine. The effect of both deficiencies and excesses of these elements alone and in combination was considered. High nitrogen consistently increased the number and size of lesions. High potash was more variable in its effect, in some experiments distinctly reducing the amount of infection and in others yielding negative results. Phosphorus and magnesium had little effect on disease development. Chlorine in excess distinctly reduced the amount of disease in some experiments. However, none of these treatments altered the character of the lesions, all of which were of the halo type of wildfire and consequently caused but little actual leaf damage.

The one conclusion from these experiments was that epidemic wildfire had not been produced by any of the methods tried. On the other hand, the failure to produce extensive leaf break-down could not be interpreted as proving the nonparasitic leaf spot conception. Rather, it appeared that the essential cause of epidemic wildfire might have been missed entirely, and later developments proved this to be the case.

The first clue as to the real predisposing factor was obtained during the summer of 1933, when it was possible for the first time to follow the entire development of an epidemic of leaf spot that was associated with a severe wind and rain storm. The storm occurred in August of that year.

RELATION OF STORM-INDUCED WATER SOAKING OF LEAVES TO EPIDEMIC WILDFIRE

FIELD OBSERVATIONS

During the storm of August 1933 numerous water-soaked areas scattered over the leaves were observed. As the strong wind and heavy rain continued, these areas increased greatly in number and size. Plants were partly tilted over by the wind, and the leaves so turned up became extensively water-soaked (fig. 2). There were noticeable differences in the amount of water soaking, depending on methods of topping and fertilization. These water-soaked areas were plainly the result of flooding of the intercellular spaces, and, following the first violent phase of the storm, they persisted for some 48 hours, until the weather began to clear. As the sun came out, many of these areas cleared up completely, but at the same time many others began to turn brown and die (figs. 3 and 4). Microscopic examinations showed abundant evidences of bacteria in the dying tissues. Isolations yielded practically pure cultures of *Bacterium tabacum*. The lesions that resulted were of the typical epidemic type of wildfire, and the disease caused great destruction throughout the tobacco sections of Maryland and Pennsylvania. As a check on the question of whether the storm itself produced this destructive leaf spot in the absence of the organism, the writer fortunately had a disease-free plot of tobacco at College Park, Md. This plot developed no leaf spot following the storm, regardless of fertilizer or topping treatment.

FIGURE 2.—Water soaking as it developed on leaves in the field following a severe wind and rain storm.

FIGURE 3.—Epidemic wildfire as it appeared on leaves a few days after they had been water-soaked as shown in figure 2. Note the irregular shape of the lesions and the absence of any halo effect. These lesions were red brown and typical of the condition often referred to as rust.

FIGURE 4.—Epidemic wildfire showing dark, zonate lesions of the type often referred to as blackfire. The color, however, is due to the fact that this was a heavy, mature leaf as compared with the immature leaf shown in figure 3. The zonate markings in this case are the indications of smaller wildfire lesions formed at an earlier date, which spread rapidly after a storm and coalesced to form the large dead area shown.

ARTIFICIAL WATER SOAKING OF LEAVES

As a result of the observations just described, the entire problem of epidemic wildfire was reconsidered. Water soaking had never been encountered in previous inoculation experiments, because the plants had always been dampened with a fine mist. Tests now showed that typical leaf water soaking could be produced with a strong water spray. By using a coarse spray and holding the nozzle about 3 feet away, it was possible to produce large water-soaked areas on leaves laid against a padded board in ½ to 2 minutes. These treated leaves recovered without injury when they were not inoculated.

INOCULATION OF WATER-SOAKED LEAVES

Inoculations made in the usual manner, except that the leaves were previously water-soaked, yielded outstanding results. Figure 5 illustrates the disease development in an experiment in which halves of leaves were water-sprayed from above until scattered areas appeared, and the entire leaves were then atomized from below with a suspension of bacteria. The tissues that were water-soaked began to break down after 64 hours, when the plants were removed from the damp chamber, and large dead areas were soon formed which coalesced to produce the dead area shown. This was still enlarging when the photograph was taken, 9 days after inoculation. Obviously, although the water-soaked areas provided the starting points, the ultimate lesions extended far beyond the borders of the original water-soaked areas.

This same sequence also occurs under natural conditions; (1) the quick break-down of the infected, water-soaked areas, which takes place during the few hours following the storm, and (2) the more gradual extension of the lesions, which continues for as much as 2 weeks. In contrast to this, the small halo lesions, on the side of the leaf that was not water-soaked (fig. 5), were not visible until about 5 days after inoculation, and after a few days they ceased to enlarge. It seems evident that the secondary extension of lesions originating with water-soaked areas can be attributed to the mass action of the tremendous population of bacteria within the host tissues. Isolations show that, whereas without water soaking the bacteria remain confined to the tissues close to the point of inoculation, with water soaking large areas of tissue may be swarming with bacteria in as short a time as 30 hours.

Since the major effect of water soaking was to increase the size of the lesions, it seemed desirable to measure this effect quantitatively. For this purpose 25 leaves were selected on plants of different ages, and one-half of each leaf was water-sprayed. Four water-soaked areas were selected on each treated half leaf and inoculated by pricking lightly in the center with a needle dipped in a suspension of bacteria. The half of each leaf not water-soaked was then inoculated similarly, and all were incubated as usual. Each experimental leaf was then outlined on a sheet of paper, and the location of the inoculations and the size of the water-soaked areas were indicated. Outlines were made of all lesions at 3, 7, and 16 days after inoculation. The results from all 25 leaves were similar, and are indicated by the detailed data from 3 representative leaves given in table 1.

FIGURE 5.—Effect of water soaking on disease development. The entire leaf was inoculated but only the right half was water-sprayed before inoculation. The lesions on the right half soon spread and coalesced, while those on the left remained small.

TABLE 1.—*Results from Bacterium tabacum inoculations on leaf with and without water soaking*

Age of leaf	Lesion no.	Size of lesions after indicated number of days					
		Water-soaked and inoculated			Inoculated only		
		3 days	7 days	16 days	3 days	7 days	16 days
		Sq. in.	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*
Young	1	0.12	0.45	[1]	0	0.06	0.06
	2	.06	.34	5.8	0	[2]	[2]
	3	.09	.90		0	.02	.02
	4	.13			0	.05	.05
Half grown	1	.06	.52	1.25	0	.06	.06
	2	.09	.75	2.18	0	.03	.03
	3	.09	.75	2.19	0	.04	.04
	4	.04	.18	1.00	0	.03	.03
Mature	1	.02	.30	2.20	0	[2]	[2]
	2	.04	.09	1.36	0	[2]	[2]
	3	.08	.50	2.24	0	.03	.03
	4	.08	.75	2.00	0	[2]	[2]
Total area killed		.90	5.53	20.22	0	.32	.32

[1] The 4 original lesions coalesced to form 1.

[2] Trace.

The figures of table 1 show clearly how it is possible with the aid of leaf water soaking to produce typical epidemic wildfire. Note that with water soaking lesions of appreciable size had formed 3 days after inoculation, while without water soaking no development was apparent at this time. After 7 days, 100 percent of infection had resulted from all inoculations, but the total leaf area destroyed was increased by water soaking 1,628 percent. After 7 days there was no further increase in the size of the lesions on the half leaves not water-soaked, but the lesions with water soaking continued to enlarge, and at the end of 16 days the increase in area killed on water-soaked leaves was 6,218 percent greater than that on leaves not water-soaked. The lesions without water soaking were of the typical harmless halo type, and with water soaking they were of the quick-forming destructive epidemic type. It is to be observed also that with water soaking leaves of all ages were highly susceptible, as indicated by the large size of lesions formed. Without water soaking the mature leaves were most resistant, though the small size of all lesions indicates a high degree of resistance for the normal leaf at any age.

EFFECT OF SIZE AND PERSISTENCE OF WATER-SOAKED AREAS ON LESION DEVELOPMENT

Under natural conditions it has been observed that the water-soaked condition of many areas disappears in a few hours, and that when this occurs epidemic wildfire does not result. Both during the extensive storm of 1933 and during a more local but equally severe one in 1934, the water-soaked condition of the areas persisted for 48 hours, and then, as the weather cleared, the break-down of tissues took place. Poured-plate isolations showed that after 15 hours the bacteria were well distributed throughout some of the water-soaked areas but were not numerous. Similar isolations after 30 hours showed an enormous multiplication in numbers. It seemed desirable to find out definitely whether large water-soaked areas tended to facilitate the formation of large lesions. The large areas persist

longer, as a rule, than the small ones, but the effect of persistence will be considered separately. In seeking to evaluate the direct effect of size of water-soaked areas, it was first shown conclusively that if large and small areas are generally inoculated by atomizing the entire leaf surface with a bacterial suspension the lesions resulting from the larger areas are much the more extensive, owing to multiple infections. It is probable, however, that under many field conditions the supply of bacteria is limited, and hence comparisons were made with large and small water-soaked areas inoculated at only a single central point. In these experiments the large water-soaked areas ranged from ¾ to 1½ inches in diameter and the small areas from ¼ to ½ inch. All inoculations were incubated 48 hours, and the lesions were measured 5 days after inoculation. The results are shown in table 2.

TABLE 2.—*Size of lesions in relation to size of water-soaked areas*

Experiment no.	Size of lesions on leaves with—					
	Large water-soaked areas			Small water-soaked areas		
	Tip leaves	Middle leaves	Basal leaves	Tip leaves	Middle leaves	Basal leaves
	Sq. in.	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*
1	1.1	1.25	1.25	0.25	0.25	1.08
2	1.08	1.25	1.56	.29	1.04	1.16
3	1.75	1.17	1.22	.30	.96	.92

Records of the check inoculations, without water soaking, are not included in table 2, because at the time data were taken they appeared merely as faint halos and no dead tissue was observed. The tendency of the large water-soaked areas to facilitate the formation of large lesions is quite apparent, and the data again point to the marked effect of the intercellular liquid in facilitating spread of the bacteria through the tissues.

The relation of persistence of the water-soaked areas to the development of epidemic wildfire was studied by water soaking leaves and then holding the plants for various periods in a saturated atmosphere. The water soaking disappeared as soon as the plants were removed to ordinary greenhouse conditions. The results, taken in terms of percentage of leaf area killed 11 days after inoculation, are presented in table 3.

TABLE 3.—*Relation of persistence of water-soaked areas to disease development*

Leaf no.	Percentage of leaf killed after 11 days when water-soaked areas were held for—			Leaf no.	Percentage of leaf killed after 11 days when water-soaked areas were held for—		
	10 hours	24 hours	50 hours		10 hours	24 hours	50 hours
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
1	20	40	35	8	15	50	80
2	50	30	35	9	40	75	75
3	20	10	85	10	15	75	85
4	10	30	30	11	60	80	95
5	10	25	25	12	5	75	85
6	10	25	25				
7	10	50	30	Average	22	47	57

Counts were made of number of lesions, and there were about as many with 10 hours' as with 24 or 50 hours' incubation. Hence, the differences in percentage of leaf area killed were the result of the much larger lesions that developed with water-soaked areas persisting 24 and 50 hours. The great increase in leaf area killed occurred between 10 and 24 hours, and a further increase from 24 to 50 hours had but little effect. The results obtained from this and similar experiments showed that for the development of the destructive epidemic type of wildfire the water-soaked areas must persist for 24 hours or more after inoculation has occurred. Under field conditions, inoculation and infection may not occur for some time after the start of the storm; in fact, many water-soaked areas never become infected, and all traces of water soaking disappear. The persistence factor consequently is very important, and, with both natural epidemics studied, water soaking persisted for about 48 hours.

EPIDEMIC WILDFIRE REPRODUCED IN THE FIELD

As a final proof of the relation of water soaking to epidemic wildfire development, this type of disease was reproduced in the field. To accomplish this, plants were sprayed during the early stages of ordinary rainstorms until water-soaked areas appeared on the leaves. Part of the sprayed plants were then inoculated with *Bacterium tabacum*, and part were held as uninoculated checks. Other plants were inoculated but not water-sprayed. Figure 6 shows epidemic wildfire that resulted from the combination of water spraying and inoculation. Figure 7 shows the healthy condition of similar plants that were water-sprayed but not inoculated. Plants inoculated but not water-sprayed developed the usual harmless halo spots.

FACTORS MODIFYING SUSCEPTIBILITY OF LEAVES TO WATER SOAKING

SURFACE OF LEAF EXPOSED

It has been shown that when leaves are held in a firm position and a strong water spray is applied extensive water soaking can be produced in one-half to 2 minutes. Under field conditions, however, leaves do not become water-soaked so readily. In fact, only the severest storms produce any considerable amount of water soaking, and slight variations in the susceptibility of the leaves result in marked differences in the amount of water soaking and hence in the severity of disease development. Observations during storms indicated that water soaking was greatly facilitated when the lower leaf surface was exposed. The susceptibility to water soaking of upper and lower leaf surfaces was measured under controlled conditions. Half of each leaf was sprayed from above and the other half from below. Table 4 gives the results from such measurements with two sets of leaves—the high-nitrogen leaves, which were very susceptible to disease, and the low-nitrogen leaves, which were very resistant. All the plants from which the leaves were taken were topped low. Even during the severe epidemic of 1933 very little disease developed in the low-nitrogen plots.

FIGURE 6.—Epidemic wildfire produced in the field by water soaking leaves with a power sprayer, and inoculating during the progress of an ordinary rain.

FIGURE 7.—Several plants only a few feet distant from those shown in figure 6. These plants were water-soaked with the power sprayer, but they were not inoculated. The water soaking disappeared completely as soon as the weather cleared.

TABLE 4.—*Relative susceptibility to water soaking of upper and lower leaf surfaces on high-nitrogen and low-nitrogen plants*

Leaf no.	Time required to water-soak indicated surface of leaf from high-nitrogen plants		Time required to water-soak indicated surface of leaf from low-nitrogen plants		Leaf no.	Time required to water-soak indicated surface of leaf from high-nitrogen plants		Time required to water-soak indicated surface of leaf from low-nitrogen plants	
	Upper	Lower	Upper	Lower		Upper	Lower	Upper	Lower
	Seconds	*Seconds*	*Seconds*	*Seconds*		*Seconds*	*Seconds*	*Seconds*	*Seconds*
1	90	45	240	180	4	105	60	360	360
2	70	45	330	270	5	110	40	300	420
3	90	45	235	160	Average	93	47	293	278

The marked difference in susceptibility to water soaking between leaves from low-nitrogen and those from high-nitrogen plants is to be noted first, and the extreme resistance of the former explains adequately their freedom from epidemic wildfire. The most rapid water soaking was secured with high-nitrogen leaves having the lower leaf surface exposed, the average time being 47 seconds. The same leaves, but with upper surface exposed, required an average of 93 seconds to water-soak.

In another experiment leaves were taken from plants given average topping and fertilization treatment. As in the previous test, half of each leaf was sprayed from above and the other half from below. However, the duration of the spray treatment was 1 minute from below and 2 minutes from above. The data were taken in terms of size of water-soaked areas (table 5).

TABLE 5.—*Relative susceptibility of upper and lower leaf surfaces to water soaking*

Leaf no.	Size of water-soaked area		Leaf no.	Size of water-soaked area	
	Lower surface sprayed 1 minute	Upper surface sprayed 2 minutes		Lower surface sprayed 1 minute	Upper surface sprayed 2 minutes
	Sq. in.	*Sq. in.*		*Sq. in.*	*Sq. in.*
1	16.9	12.26	5	30.5	18.8
2	24.2	15.0	6	25.2	9.7
3	12.5	4.5			
4	10.5	7.5	Average	20.0	11.3

A comparison of average results shows that a 2-minute treatment of upper surfaces produced 11.3 square inches of water soaking, while a treatment half as long of lower surfaces gave 20 square inches. Similar experiments have been conducted with many lots of leaves. Lower-surface exposures of equal duration have yielded water-soaked areas as much as five times as large as those obtained with upper-surface exposures. Hence it may be concluded that if leaves are so turned that the under surface is exposed to the rain, water soaking is greatly facilitated.

Earlier in this study it was shown that the normal leaf, not water-soaked, is more readily infected by applying the inoculum to the

lower leaf surface, and that the upper surface of mature leaves is especially difficult to infect. In view of this, it seemed advisable to consider the effects of different combinations of water-spray treatments of upper and lower leaf surfaces with inoculations, as shown in table 6. This experiment was repeated several times with similar results.

TABLE 6.—*Effect of different combinations of water-spray and inoculation treatments on disease development in old and in young leaves*

Leaf surface treated		Old leaves		Young leaves	
Water-sprayed	Inoculated	Average number of lesions	Average percentage of leaf area killed	Average number of lesions	Average percentage of leaf area killed
None	Upper	1	0	24	1.5
Upper	do	31	47	83	50
Lower	do	65	80	200+	80
None	Lower	6	1	85	3.5
Lower	do	118	100	200+	90
Upper	do	69	87	200+	80

In comparing results obtained with old and with young leaves, it is to be noted that, although all leaves were equally water-soaked in the beginning and saturated air conditions were maintained during incubation, the water-soaked areas were always retained more completely by the more mature leaves. This difference is associated with slightly higher values for percentage of leaf area killed in the case of the mature leaves. The chief point brought out by table 6, however, is that there is little relation between susceptibility to infection of the normal leaf and the development of epidemic wildfire. The old leaves that were infected with difficulty when not water-soaked were destroyed by the disease after water soaking even more completely than were the young leaves that were readily infected. Table 6 shows also that, regardless of which leaf surface was water-sprayed and which was inoculated, all developed the epidemic type of disease; the minimum of leaf area destroyed was 47 percent and the maximum 100 percent. Without water soaking, the same inoculations produced only the halo type of wildfire, and the minimum and maximum figures for area killed were 0 and 3.5 percent, respectively.

LEAF INJURIES

In the field, after storms, water-soaked areas were frequently observed surrounding old wildfire lesions. Similar water-soaked areas were noted about fresh mechanical injuries, but old injuries or insect punctures had no effect. Experiments under controlled conditions confirmed these observations and showed conclusively that both fresh mechanical injuries and wildfire lesions of any age materially reduce leaf resistance to water soaking. Toward the latter part of the summer medium- to small-sized wildfire lesions often are very numerous, and their function in supplying bacteria for further disease development is obvious. The data in table 7 show their importance in increasing susceptibility of leaves to water soaking. In this experiment pairs of adjoining healthy and diseased leaves from the same plant were compared.

TABLE 7.—*Effect of old wildfire lesions on the susceptibility of leaves to water soaking*

Leaf pair no.	Size of water-soaked area of leaf with—				Leaf pair no.	Size of water-soaked area of leaf with—			
	Upper surface sprayed 1½ minutes		Lower surface sprayed ¾ minute			Upper surface sprayed 1½ minutes		Lower surface sprayed ¾ minute	
	Diseased leaf	Healthy leaf	Diseased leaf	Healthy leaf		Diseased leaf	Healthy leaf	Diseased leaf	Healthy leaf
	Sq. in.	*Sq. in.*	*Sq. in.*	*Sq. in.*		*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*
1	16.5	3.2	7.75	7.75	7	5.5	3.2	6.5	2.0
2	8.0	4.0	7.3	6.7	8	12.0	4.5	8.3	9.0
3	5.5	.75	5.75	1.0	9	19.0	12.5	17.9	18.6
4	6.25	6.25	4.0	4.2	10	12.0	2.6	11.0	12.0
5	15.0	7.5	7.7	3.4					
6	11.0	5.5	12.5	12.5	Average	11.1	5.0	8.9	7.7

The 10 pairs of leaves used in this experiment were selected with special care to insure that they were as nearly alike as possible, except for the presence or absence of wildfire lesions. These lesions, however, were neither numerous nor large. The outstanding development was the marked increase in susceptibility of upper leaf surfaces to water soaking, as a result of the presence of disease lesions. This correlates with the field observation that diseased leaves often show extensive water soaking while adjoining healthy leaves are but little affected.

Indirectly these experiments indicated that the intercellular water is driven in from the outside rather than exuded from the cells, and that many wildfire lesions which appear inactive are probably enlarging slowly, since otherwise they should behave in the same manner as old mechanical injuries.

LEAF MATURITY

When tobacco plants are either topped high or not topped at all, the basal leaves may be mature while the tip leaves are yet very young. These differences in leaf maturity have been associated with marked differences in the susceptibility of the leaves to water soaking and to epidemic wildfire, and, while nutrition affected the total amount of disease, it did not alter the base-tip relation. Thus, field counts following the 1933 epidemic showed in the low-nitrogen plots averages per plant of one basal leaf killed, the next two or three leaves severely injured, and from there to the tip a rapid decrease in injury to a bare trace. The upper three-fourths of these plants showed abundant infection, but the lesions were almost entirely of the harmless halo type. Adjacent plots that received no potash averaged four basal leaves killed and the next four or five severely injured, only the tip quarter of the plant escaping injury. These differences in wildfire injury on different portions of the same plant were clearly associated with differences in water soaking. The areas were much larger on basal leaves and persisted much longer.

Susceptibility to water soaking of different leaves from the same plant was measured in the usual manner. Table 8 shows the results from one experiment in which a half-minute treatment of the lower leaf surface was used.

TABLE 8.—*Relative susceptibility to water soaking of basal, middle, and tip leaves*

Plant no.	Area water-soaked			Persistence of water-soaked condition		
	Basal leaves	Middle leaves	Tip leaves	Basal leaves	Middle leaves	Tip leaves
	Sq. in.	*Sq. in.*	*Sq. in.*	*Hours*	*Hours*	*Hours*
1	27.5	10.8	0.0	5.5	0.25	0.0
2	18.3	1.0	.2	2.75	2.25	1.0
3	12.8	1.0	.3	5.0	2.5	.5
4	21.3	2.3	.2	4.5	2.0	1.0
5	13.0	1.3	.4	4.0	1.0	.75
6	11.5	1.5	.2	4.75	1.25	.75
Average	17.4	2.98	.22	4.42	1.54	.67

The results given in table 8 show clearly that from the base to the tip of the plant the size of the water-soaked areas decreases rapidly, as does also the length of time that the water-soaked condition persists. These differences are quite adequate to explain the observed difference in disease development. Additional studies of the relation of leaf maturity were conducted by inoculating plants of different ages in the greenhouse and by making successive plantings in the field. In such tests the older plants were severely injured by the epidemic type of wildfire, while the young plants, though freely infected, suffered but little injury.

The data given in table 8 were secured by water spraying lower leaf surfaces. Treatments applied to the upper leaf surfaces yielded results quite as marked. Thus in one experiment, in which a 45-second treatment was used, mature leaves developed large water-soaked areas that persisted for an average period of 5 hours and 50 minutes. The much smaller areas on the immature leaves persisted for an average period of 37 minutes. In conclusion, it is to be noted that while young leaves tend to escape water soaking, and hence disease damage, yet under very severe storm conditions both young and old leaves may become heavily water-soaked. When this happens, there is no difference in the degree of subsequent disease injury.

HEIGHT OF TOPPING

The height at which plants are topped has a consistent and marked effect on their susceptibility to leaf spot. It is probable that the primary effect of topping is on leaf maturity. Thus, as has been pointed out in the discussion of leaf maturity, plants that either are not topped or are topped high bear old leaves at the base and young leaves at the tip. Topping hastens the maturity of the tip leaves that remain, and the lower the plants are topped the greater is the tendency for the remaining leaves to mature together. The effect of such low topping on disease susceptibility is illustrated by a greenhouse experiment in which all leaves were uniformly water-sprayed and inoculated; the results are summarized in table 9.

TABLE 9.—*Effect of topping on leaf spot injury*

Location of leaves on plants	Leaf area killed		
	Low-topped	High-topped	Not topped
	Percentage	*Percentage*	*Percentage*
Base	79.3	67.9	78.0
Middle	81.7	36.6	43.4
Tip	75.4	9.4	7.1

The low-topped plants averaged 6 leaves, the high-topped 13 leaves, and those not topped 16 leaves. Infection was abundant on all leaves, and the differences in percentage of leaf area killed were due to differences in size of lesions. It is noteworthy that all the leaves of the low-topped plants responded alike to the disease and were highly susceptible, while in the other plants there were marked differences in susceptibility between the basal and the tip leaves.

The effect of topping on disease was equally marked in the greenhouse and in the field. The data collected from the field plots at Upper Marlboro, Md., following the epidemic of 1933, were of special interest.

TABLE 10.—*Influence of topping on wildfire development in the field in 1933*

Location of leaves on the plants	Plants topped low			Plants not topped		
	Average number of lesions	Diameter of lesions [1]	Area killed	Average number of lesions	Diameter of lesions	Area killed
		Inches	*Percent*		*Inches*	*Percent*
Base	800+	0.44	75	800	0.13	10
Middle	650	.28	40	650	.09	5
Tip	600	.22	25	375	.06	1

[1] The lesions measured had space enough for full development; many lesions were so close together that they soon coalesced.

The low-topped plants averaged 13 leaves; the plants that were not topped, 25 leaves. The figures for the average number of lesions per leaf are given because they show clearly that all leaves were freely infected, and the differences in disease loss were the result of the much larger size of the lesions on the leaves of the plants topped low. It is to be noted that in this field experiment even the basal leaves of the plants that were not topped suffered but little injury.

Field observations indicated that the differences in disease susceptibility induced by topping were the result of differences in susceptibility to water soaking, which either facilitated or inhibited the development of the epidemic type of wildfire. Figure 8 illustrates this effect of topping on type of disease developed. To measure susceptibility to water soaking under actual field conditions, selected plants were treated with a power spray during the course of an ordinary rain, and counts of number of water-soaked areas persisting were made when the weather began to clear, 19 hours later. The average number of areas on low-topped plants was 15.3 per leaf; on high-topped plants, 0.2 per leaf. In another test of susceptibility to water soaking, 21 leaves were picked from low-topped plants and an equal number

from plants that were not topped. All were subjected to a uniform water-spray treatment, and data were taken on both size and persistence of water-soaked areas. The comparative figures for water-soaked areas were as follows. For size: Low-topped, 15.45 square inches; high-topped, 7.15 square inches. For persistence: Low-topped, 5.8 hours; high-topped, 3.9 hours. The water spray in this experiment was applied to the lower leaf surface. Previous experiments had indicated that where differences in susceptibility to water soaking were involved these differences tended to be greater with

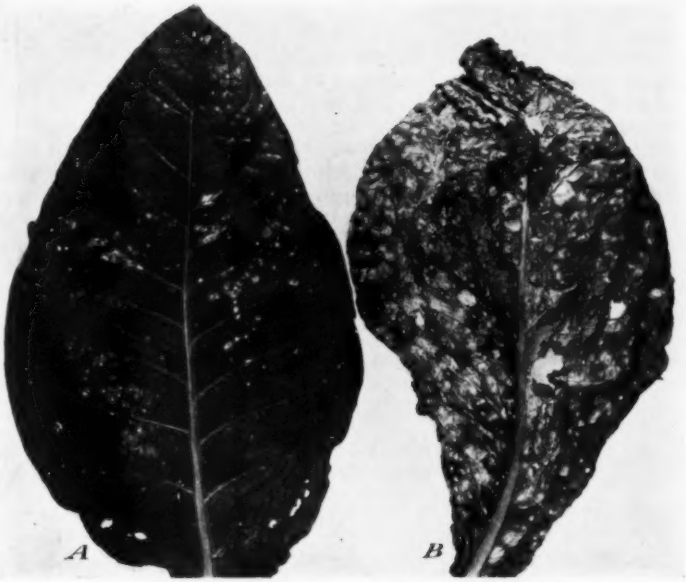

FIGURE 8.—*A*, Halo wildfire on a plant that was not topped. *B*, Epidemic wildfire on an adjacent low-topped plant. Leaves on plants that are not topped water-soak with difficulty and, except under the most severe storm conditions, are but slightly injured. Leaves on low-topped plants water-soak easily and the areas persist, hence their susceptibility to the epidemic type of disease.

upper leaf surfaces. The results of an experiment summarized in table 11 tended to support this view.

TABLE 11.—*Influence of topping on the susceptibility of leaves to water soaking*

Leaf no.	Water-soaked areas				Leaf no.	Water-soaked areas			
	Lower surface sprayed 1 minute		Upper surface sprayed 2 minutes			Lower surface sprayed 1 minute		Upper surface sprayed 2 minutes	
	Low-topped	Not topped	Low-topped	Not topped		Low-topped	Not topped	Low-topped	Not topped
	Sq. in.	*Sq. in.*	*Sq. in.*	*Sq. in.*		*Sq. in.*	*Sq. in.*	*Sq. in.*	*Sq. in.*
1	14.3	12.0	10.9	7.3	6	24.0	8.3	6.3	2.5
2	22.3	10.0	11.8	2.5	7	19.3	11.1	6.0	1.3
3	7.7	4.0	2.8	1.3	8	10.0	6.7	9.5	.0
4	5.3	1.0	4.3	1.0					
5	30.0	12.3	15.3	7.5	Average	16.6	8.2	8.4	2.9

The data presented in table 11 show that when the water spray was applied to the lower leaf surface the areas produced were twice as large on leaves from low-topped plants; when the upper surfaces were treated, the areas on leaves from low-topped plants were three times as large. The data show also the wide variation in size of water-soaked areas produced on supposedly similar leaves. Thus on leaves from the low-topped plants the range was from 5.3 to 30.0 square inches. Wide variations such as this have been the rule in the field, where they were first assumed to be due entirely to unequal exposure. Evidently leaves that are close together and look alike may yet possess differences sufficient to have a marked effect on the amount of water soaking. This explains why apparently similar leaves may be either slightly or severely injured by the disease.

Before concluding the discussion of topping, attention should be called to several modifying factors that are operative under field conditions and that tend to increase the differences previously noted. In the first place, leaves on low-topped plants are stiff and brittle while leaves on plants not topped are thinner and more pliable. Consequently, leaves on low-topped plants offer a much firmer resistance to wind and rain, and, when they do bend, mechanical injuries are frequent. In the controlled experiments all leaves were held against a padded board and much care was taken not to injure them. This procedure eliminated the field conditions mentioned. A second field factor, facilitating the water soaking of leaves on low-topped plants, was the tendency of these leaves to turn over in the normal course of growth, and so to expose the under surface. The counts from field plots given in table 12 indicate the comparative number of upturned leaves found under different fertilization and topping treatments.

TABLE 12.—*Topping in relation to number of upturned leaves* [1] *on plants grown with various quantities of nitrogen*

Fertilizer treatment	Upturned leaves on—	
	Low-topped plants	Plants not topped
	Number	*Number*
No nitrogen	3	0
Regular nitrogen	12	7
High nitrogen	56	26

[1] Total for 40 plants in each case.

The counts of leaves turned up were the totals in each case for 40 plants. Since there were twice as many leaves on the plants that were not topped, the actual differences, in proportion to total number of leaves, were much greater even than indicated in table 12. The greater susceptibility of upturned leaves to water soaking results in these being the first to develop epidemic wildfire, and it is interesting to note that low topping and high-nitrogen fertilization, the combination that produces maximum leaf susceptibility, also produces the maximum of upturned leaves. This factor, then, has an appreciable effect in making a susceptible planting even more susceptible.

FERTILIZATION

In the nutrition experiments at Upper Marlboro, the development of wildfire has been consistently modified by variations in fertilization. The plots most susceptible to disease were those that were topped low and received a high-nitrogen fertilizer, those receiving no potash, and those receiving no fertilizer at all. Resistance was clearly correlated with low-nitrogen and high-potash fertilization. Figure 9 shows the marked effect of fertilization on disease development. Just as in the case of other factors, these differences have been associated with modifications in leaf-water relations, by both field observation and controlled experimentation. Table 13 clearly shows the marked effects of fertilization on disease development.

TABLE 13.—*Wildfire development as modified by fertilization, Upper Marlboro, Md., 1933*

Fertilizer treatment	Average number of lesions per leaf	Average number of leaves per plant				Estimated total damage
		Killed	Severely injured	Slightly injured	Not injured	
						Percent
No fertilizer	(1)	3	7	3	0	80
No potassium	850	2	5	6	0	55
No phosphorus	350	0	2	8	4	20
No nitrogen	150	0	0	4	10	3
Complete fertilizer	500	0	3	10	4	25

[1] Lesions coalesced.

Table 14 shows the susceptibility to water soaking of leaves from the above-mentioned plots. The values in each case are averages for five leaves, and the experiment was duplicated with similar results.

TABLE 14.—*Susceptibility of the leaves to water soaking as affected by fertilization*

Fertilizer treatment	Average [1] size of water-soaked areas	Average [1] persistence of water-soaked areas	Fertilizer treatment	Average [1] size of water-soaked areas	Average [1] persistence of water-soaked areas
	Sq. in.	*Hours*		*Sq. in.*	*Hours*
No fertilizer	21.3	1.9	No nitrogen	3.9	0.2
No potassium	22.8	2.75	Complete fertilizer	12.58	1.3
No phosphorus	11.5	1.2			

[1] Average for 5 leaves.

The correspondence between susceptibility to water soaking, as indicated either by the size or persistence of water-soaked areas (table 14), and disease susceptibility, as indicated by the percentage of estimated total damage (table 13), is so apparent as to require no comment. The only variation from the expected was that, with no fertilizer, water-soaking values were not so high as would be anticipated. Additional evidence that fertilization modifies disease development through its effect on water relations may be seen in table 4, where susceptibility to water soaking of leaves from high- and low-nitrogen plants is compared. The former were disease-susceptible and easily water-soaked, the latter disease-resistant and difficult to

water-soak. The effect of high-potash fertilization in retarding disease development and of low potash in promoting disease development has been mentioned, and this, too, is correlated with water

FIGURE 9.—Representative half leaf (*A*) from a no-nitrogen plot and (*B*) from a no-potash plot. The former is resistant to water soaking, the latter susceptible. The epidemic wildfire shown in *B* followed the severe August storm of 1933.

relations. Thus it was found that in the absence of potash the average size of the water-soaked areas was 28.4 square inches and their average persistence 3.2 hours, whereas when potash at the rate of

240 pounds per acre was used the average size of the water-soaked areas was only 5.8 square inches and their average persistence 0.1 hour.

In conclusion, however, it is to be noted that these marked effects from fertilization were obtained at Upper Marlboro, with a very light sandy soil, and that the treatments indicated had been repeated for a number of years. Experiments elsewhere, with more fertile soils and with the different treatments applied to but a single year's crop, failed to show marked differences in plant growth, in disease development, or in susceptibility to water soaking, except in the case of nitrogen. Hence, while it may be safely concluded that excessive nitrogen applications will generally produce increased susceptibility to wildfire, both the nature of the soil and previous fertilization evidently will determine the effectiveness of potash. For example, in Pennsylvania the soil type is such that plants absorb but little potash, regardless of the amount applied.

DISCUSSION

This investigation, which was undertaken to elucidate the etiology of the destructive type of leaf spot that attacks tobacco in the Maryland-Pennsylvania area, resolved itself into a study of the relation of storms to the development of epidemic wildfire. The relation of storms to the development of destructive wildfire epidemics had been reported many times and had been explained on the basis of dissemination. Thus Chapman and Anderson (*4, p. 71*) early showed that the organism could be readily spread by rain spattering, and their explanation of storm effect was as follows:

> It has been noted by all investigators of the disease and by tobacco growers that rapid spread invariably follows heavy rains. When the raindrops fall on the diseased spots, the bacteria float out into the water and successive drops splash them to other leaves of the same plant or neighboring plants. If the rain is accompanied by wind, the drops are carried farther and the spread is greater to the windward of diseased plants. * * * These two agents (wind and rain) are undoubtedly the most potent of all the factors involved in dissemination.

While this conception was generally accepted, certain other workers offered different hypotheses. Thus Johnson and Fracker (*11, p. 13*) say:

> Storms, especially beating rains, however, have a very important relation to wildfire in that they favor infection to a high degree. The bacteria are often unable to infect leaves except through slightly wounded tissue, such as may be produced by beating rain, although with rapidly growing tobacco, moisture in itself often suffices for considerable infection. The facts are, generally, that heavy infection and damage practically depend on storms or continued rains.

Still another interpretation of storm effect was given by Clinton and McCormick (*7, pp. 387, 388*) as follows:

> The greatest injury comes just after the disease spreads over the plants during the period of wet weather and the sun suddenly shines again. The halo spots then turn to brown irregular burn-like areas. This transformation often takes place quickly and so probably is largely a mechanical injury to the badly infected tissues.

The statements given in the last two quotations appeared in 1922 and have not been referred to since then. More recently Böning (*2, pp. 12–18*) has advanced the hypothesis that rains affect disease development by modifying plant nutrition. He suggested that during dry weather nitrates accumulate in the surface soil, and that the rains

then wash the nitrates down to where the roots can absorb them. In this manner the nitrogen intake is increased and the leaves are made more susceptible.

The negative results reported in the first part of this paper show quite conclusively that the development of epidemic wildfire cannot be adequately explained by dissemination, leaf injury, or nutrition. Inoculations, in which these and all other known environmental and plant factors were widely varied, always resulted in the formation of small dead areas, rarely more than one-fourth inch in diameter, which were surrounded by yellow halos. Even large numbers of these halo lesions caused but little actual leaf damage.

The first definite clue to the factor responsible for the development of the destructive epidemic type of wildfire was the discovery that during very severe rain and wind storms numerous water-soaked areas appeared scattered over the leaves. These were external evidence that the intercellular spaces below were flooded. So far as the writer is aware, the only previous observation of these water-soaked spots was made by Valleau and Johnson (*15*), who associated them with what they considered to be nonparasitic blackfire. The writer found that these water-soaked areas facilitated invasion by the bacteria but that by far their most important effect was in facilitating the spread of the bacteria through the leaf tissues after infection had occurred. This penetration was so rapid that large areas of diseased leaf began to wilt and die after 48 hours. The death of the diseased tissues was most rapid after the sun appeared, just as suggested by Clinton and McCormick (*7*), but only because the sun hastened the necrosis. Following this initial rapid-development phase, the bacteria were able to invade and kill new tissues for a period of 10 to 14 days, and this resulted in a secondary and more gradual phase of lesion enlargement.

It is of interest now to consider why the lesions of epidemic wildfire differ so materially in appearance from those of halo wildfire. The conspicuous feature of the latter is the yellow halo surrounding the small central dead area, and the confusion regarding the cause of epidemic wildfire was due (1) to the rapid development of the lesions, (2) to their large size, and (3) to the fact that they showed little or no halo effect around the margins. The halo is caused by the toxin excreted by the bacteria diffusing out ahead of the organism into adjacent living cells and destroying the chloroplasts, as has previously been described (*6*). Hence halo lesions result when the bacteria remain alive for some time in an area but are unable to rapidly invade and destroy the adjacent cells, thus giving the toxin time to diffuse out ahead. With the aid of water soaking, however, tissue invasion proceeds at such a rate that the adjoining tissues are killed before there is time for the development of halos. Thus the appearance of halo lesions, instead of being proof of parasitic activity, is really proof that bacterial invasion has been checked successfully by the host. In this connection it is to be recalled that the *Bacterium tabacum* toxin is able to produce halo spots on inoculation into the leaves of many plants, but the bacteria are able to parasitize only tobacco. It now appears that they are able to successfully parasitize tobacco only under very special conditions, i. e., when the resistance of the leaf has been broken down by water soaking. Hence we have the unusual situation of a host that is highly resistant to the parasite under all

ordinary conditions but which can be rendered highly susceptible through the action of beating rain. In addition to this major storm effect on host susceptibility, it is not questioned that spattering water aids greatly in the dissemination of the organism, and that this dissemination under favorable conditions for infection is a necessary factor in the development of the leaf spot epidemic. Growers have been inclined to attribute leaf spot damage directly to storms, but this is proved to be incorrect by the fact that, in the absence of the organism, leaf spot does not develop, regardless of storm severity.

It has also been shown that such practices as high-nitrogen fertilization and low topping do not in themselves make plants susceptible to epidemic leaf spot but that by making the leaves more susceptible to water soaking they act indirectly. High humidity, also, does not produce epidemic wildfire, but high humidity following water soaking helps to maintain the water-soaked areas, and epidemic leaf spot development requires that these areas persist at least 24 hours. Various other factors that affect disease development in the field have also been examined and have been found to exercise these effects by modifying water relations in the leaves. Thus, during epidemics of wildfire the leaves on the windward side of plants were most severely injured, and this was supposed to be due to the greater exposure of these leaves to infection. What actually happens is that the wind bends the plants and turns up the leaves on the windward side so that under surfaces are exposed. These leaves then become heavily water-soaked, while leaves on the leeward side, with upper surfaces exposed, do not water-soak so readily.

The mechanics of water soaking has not been investigated, but certain suggestive evidence may be mentioned. In the first place, leaves do not readily water-soak when dipped in water or when exposed to a gentle spray, but a hard spray is very effective, indicating that it is not merely water, but forcibly applied water that is required. It is possible that beating rain might alter the permeability of interior cells and cause them to excrete liquid into the spaces. However, there are indications that the water may come from outside. For example, disease lesions and fresh breaks in the leaf surface are soon surrounded by water-soaked tissues, suggesting that the water was driven in through the openings thus afforded. The structure of the leaf indicates that the lower leaf surface would be more easily penetrated than the upper. The question of how low topping and high-nitrogen fertilization make leaves more susceptible to water soaking has not been studied, other than to note that these treatments modify leaf structure materially. These problems can be definitely solved only by further investigation.

It has been demonstrated that water soaking favors rapid spread of the bacteria through the leaf tissues, and this raises the question as to how this spread takes place. Hill (*10*) has reported that *Bacterium tabacum* moves in the form of a zoogloea, which would imply a rather slow penetration. It is probable, however, that he worked with the usual halo type of lesion. The writer has prepared sections of infected water-soaked tissues, and found the bacteria widely scattered through the intercellular spaces, with no indication of zoogloea. Hence it seems likely that under epidemic conditions the bacteria spread along the liquid pathways as free-swimming cells,

and that this is the reason for the resulting rapid and extensive disease development.

In view of the dependence of epidemic wildfire on storms, the question naturally arises as to whether some other plant diseases may not be similarly influenced. A search of the literature has failed to disclose any evidence that the storm water-soaking relation has been observed by others, but several investigators have noted the role of intercellular liquid in facilitating infection and disease development. Thus Riker (*13*) found that, when he inoculated stems with *Bacterium tumefaciens* by wounding, the tissues around the wounds became water-soaked. The resulting galls never extended beyond the water-soaked areas. Similarly, Meier (*12*) reports that infection with *Bact. campestre* was restricted to the margins of the leaves because only there did the hydathodes provide a liquid pathway for the bacteria. The writer, however, has previously reported (*5*) that infection with *Bact. campestre* occurs at any point on the leaf under some conditions, and that such epidemic outbreaks of black rot were preceded by the appearance of numerous water-soaked areas scattered over the leaf. The water-soaked areas in this case were formed during warm humid nights, without the aid of storms. The subsequent development of black rot lesions followed the course here described for epidemic wildfire.

It seems likely that further investigation will show that the development of water-soaked tissues as the result of storms or other causes is an important factor in the epidemiology of a number of diseases. Evidence not yet published shows that this is true of another tobacco leaf spot disease, blackfire, caused by *Bacterium angulatum*.

Several hypotheses advanced by other workers to explain resistance and susceptibility to wildfire are quite different from the conception herein presented, which is, briefly, that the normal tobacco leaf is highly resistant to invasion but that by water soaking it is rendered highly susceptible, and that various cultural and nutritional factors do not make cells more or less easy to attack but do make tissues more or less easy to water-soak. Dufrénoy (*8*) attributes to the host tissues ability to set up a definite defense mechanism and suggests that the lesions cease to spread because of phenolic compounds that tre formed in the cells surrounding the lesions. However, the writer finds that under field conditions the tissues adjoining old lesions are very likely to be first invaded when a subsequent epidemic develops. This is correlated with the fact that the tissues adjoining old lesions are easily water-soaked; there is no indication that they are fundamentally more or less susceptible. Böning (*3*) associates wildfire susceptibility and resistance with nutritional balance in the leaves but offers no satisfactory explanation of how so-called unbalanced leaf nutrition would be effective in inducing resistance and susceptibility.

The results obtained by the writer do not indicate that tobacco-leaf tissues differ to an important degree in their inherent susceptibility or resistance to attack by *Bacterium tabacum*, since if intercellular liquid is present all are about equally and completely susceptible and if it is not present all are resistant. It seems, consequently, that apparent leaf resistance is merely a matter of disease escape, with water relations as the immediate deciding factor. Also, the assumption generally made that susceptibility to infection indi-

cates susceptibility to the epidemic type of disease does not hold. Young leaves were susceptible to infection but resistant to epidemic wildfire in the field because they were not readily water-soaked.

In conclusion, it should be noted that, theoretically, effective wildfire control can be accomplished (1) by eliminating the organism, (2) by protecting the plants from storms (as is actually done on a small scale where crops are grown under cloth), or (3) by securing resistance either directly or through resistance to water soaking. At present, with the organism generally distributed in most fields throughout the Maryland-Pennsylvania area, it may be safely predicted that destructive wildfire epidemics will follow severe late summer wind and rain storms, and when such epidemics occur the damage to the plants will be modified by the various factors that increase or diminish leaf resistance to water soaking.

It is believed that the experiments herein reported adequately explain how *Bacterium tabacum* is able to cause the usual and relatively harmless halo lesions and also the destructive epidemic type of the disease and show that there is no evidence of the existence of a similar nonparasitic leaf spot.

SUMMARY

Tobacco leaves are readily infected by *Bacterium tabacum*, but under ordinary conditions invasion is limited to small areas. The usual result of infection is a dead spot, one-fourth of an inch or less in diameter, surrounded by a yellow halo. These halo lesions do little damage, but have been regarded heretofore as typical wildfire.

The destructive epidemic type of wildfire is characterized by large lesions that develop very quickly and show little or no halo effect. The question has been raised as to whether this type of disease might be wholly or partly nonparasitic in nature.

Inoculation experiments designed to reproduce the epidemic type of lesion by varying the nutrition of the plant through fertilization and topping resulted only in the halo type of lesion. In other experiments consideration was given to age of leaves, methods of inoculation, and incubation conditions, particularly temperature and humidity, but the lesions obtained were all of the halo type.

Observations during storms showed that the tobacco leaves develop water-soaked areas owing to the flooding of intercellular spaces. When these areas become infected the bacteria multiply and spread through the tissues with extraordinary rapidity, and the epidemic type of lesion results.

Without water soaking, about a week was required for the development of the small halo lesions; but with water soaking, large epidemic-type lesions were produced in 48 hours. In controlled inoculations, water soaking increased the size of lesions by more than 6,000 percent.

Large water-soaked areas tended to produce large disease lesions.

Persistence of water-soaked areas for 24 hours or more was essential for epidemic disease development.

With the aid of a power sprayer to water-soak the leaves, it was possible to reproduce the typical epidemic wildfire under field conditions. Susceptibility of leaves to water soaking, however, was found to be modified by many factors, and these differences account for wide differences in disease damage.

Exposure of the lower leaf surface greatly facilitated water soaking, as did also the presence of fresh mechanical injuries or wildfire lesions of any age.

Mature basal leaves became water-soaked more readily than did young tip leaves on the same plant.

Low topping increased susceptibility of the leaves to water soaking, while topping high or not topping at all had the opposite effect.

High-nitrogen and low-potash fertilization both increased susceptibility of the leaves to water soaking.

There was little relation between susceptibility to infection of the normal leaf and the development of epidemic wildfire. Mature leaves were infected with difficulty when not water-soaked, but after water soaking they were more severely damaged than young leaves that were readily infected.

Since all types of leaves were highly resistant to bacterial invasion when not water-soaked and highly susceptible when water-soaked, it is concluded that susceptibility and resistance to *Bacterium tabacum* is primarily a question of water relations, and that the halo type of wildfire is the response to infection of the normal leaf and the epidemic type of wildfire is the response of the leaf after its resistance has been broken down by water soaking.

Epidemic wildfire in the Maryland-Pennsylvania area, consequently, is caused by *Bact. tabacum*, and its occurrence is conditional on storms of sufficient severity and duration to produce and maintain water-soaked areas on the leaves. The amount of water soaking, and hence the amount of disease damage, is modified by prevailing cultural and fertilization practices; low topping in combination with high-nitrogen fertilization, which is the practice in Pennsylvania, favors maximum disease development.

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BEHAVIOR OF THE ORDINARY TOBACCO MOSAIC VIRUS IN THE SOIL[1]

By ISMÉ A. HOGGAN, *assistant professor of horticulture*, and JAMES JOHNSON, *professor of horticulture, University of Wisconsin, and agents, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

It is fairly evident that control of the ordinary tobacco mosaic disease (*tobacco virus 1*) (*4*)[2] may be accomplished with considerable success through the application of all of a number of precautionary measures that have been recommended for the purpose. These measures are directed chiefly against introduction of the virus into the field from various sources where it may be harbored for long periods, and also against further dissemination of the disease once it has gained entrance into the crop. The means by which the virus, once introduced, is spread in the field are comparatively well understood and may be prevented in considerable degree. The relative importance of the various sources of initial infection is, however, not so clear. The multiplicity of ways in which the virus may persist for prolonged periods apart from the living tobacco plant renders the problem a complex one, requiring intensive study in its various phases. One phase of the problem in particular need of greater consideration than has been accorded to it relates to the harboring of the virus in the field soil.

The present paper deals with the results of laboratory and greenhouse studies on the persistence and inactivation of the ordinary tobacco mosaic virus in the soil under various conditions. These investigations have shown that the virus may survive in soils for periods of 1 year or more, but that it is subject to relatively rapid inactivation under certain natural conditions. This inactivation is not sufficient, however, to eliminate soil infestation as a factor in tobacco mosaic infection, the more practical aspects of which it is proposed to discuss in a later contribution from this laboratory.

EARLIER INVESTIGATIONS

A brief survey of the early literature dealing with overwintering of the tobacco mosaic virus is presented in a previous paper from this laboratory (*6*). Attention is called to the fact that, whereas the earlier observers of tobacco mosaic believed the soil to be a significant source of infection, the later trend of opinion was away from this conception and in favor of overwintering of the virus in perennial hosts, with subsequent dissemination by aphids. The latter hypothesis has now been practically eliminated, however, so far as ordinary tobacco mosaic is concerned (*2*). More recently, considerable emphasis has

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[2] Reference is made by number (italic) to Literature Cited, p. 294.

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been placed on infection occurring during cultural operations through the use of contaminated tobacco by workers (*7*, *9*).

In 1929 Johnson and Ogden (*6*) presented data showing that the tobacco mosaic virus overwintered naturally in the soil under Wisconsin conditions, and that tobacco transplanted to heavily infested soils developed much higher percentages of infection than did similar plants set in virus-free or slightly infested soils. While other sources of natural infection were recognized, such as tobacco or tobacco refuse, transferred by man, animals, or wind to seedbed or field, soil infestation was regarded as being of major significance.

As a result of various tests performed in North Carolina, Lehman (*7*) also considered soil infestation to be responsible for some direct infection of the tobacco crop, though in his trials the proportion of plants becoming diseased was too low to account for the high percentages of tobacco mosaic infection common to that section.

The wide range of conditions under which tobacco is grown may be expected to influence the origin and amount of mosaic infection in different districts. However, it appears clear that the data now available are not sufficient to permit definite conclusions to be drawn as to the relative importance of the various sources of infection in the epidemiology of the disease.

MATERIALS AND METHODS

The soils in which the behavior of the tobacco mosaic virus has been studied were chiefly "tobacco" soils. Some of these were Wisconsin soils, obtained in relatively large amounts from various parts of the State. In addition, representative smaller lots, up to three in number, were obtained from each of the tobacco-growing States through the courtesy of local, State, or Federal workers, to whom thanks are due. Several other types of soil were also used, including Superior red clay, peat, greenhouse compost, some very acid soils, and sand, so that an almost complete range was available with respect to physical properties, chemical fertility, and reaction.

All soils used are listed in table 3 under the name of the State from which they were obtained. Where lots were obtained from more than one locality in a State, they are distinguished by addition of a letter indicating the particular locality or other distinctive feature. For example, Tennessee (S) soil came from Springfield, Tenn., and Wisconsin (E) soil from Edgerton, Wis. Where more than one lot of soil was obtained from the same locality, designation is made by number, i. e., South Carolina (1), (2), and (3). The soils are listed in the table in order of their total colloidal content. The type to which each soil belongs is also indicated, together with certain other pertinent information.

The material used as soil inoculum was collected mainly from tobacco plants infected with *tobacco virus 1* in the field in September 1934. About 12 liters of juice were extracted from diseased leaves and suckers and stored in a refrigerator at 5° C. until needed for use. In addition, considerable quantities of diseased roots, stubble, stalks, sucker stems, and leaves were cut up into small portions of the required size, the leaves in very small pieces, the other parts in sections about 1½ inches long. These were thoroughly air-dried and stored in a dry cupboard at room temperatures to serve as stock material fairly uniform in virus concentration.

White enamel refrigerator pans of about 5-kg capacity, with covers, were selected as containers for most of the experiments. These pans were chosen, apart from their convenience in handling, chiefly because they allowed approximate reproduction of the conditions of soil aeration and moisture existing at soil depths of about 4 to 8 inches. Soil stored in the covered pans lost moisture only very slowly. The usual procedure was to place 2 kg of air-dried soil in each pan and to bring the soil to approximately one-third of the water-holding capacity by adding 200 cc of virus extract diluted to 1 to 1 with water, together with as much additional water as was required. Thus, the effective dilution of the virus in the soil lots was regarded as being about 1 to 20. After standing for sufficient time to allow the moisture to be absorbed by the soil, the contents of the pan were turned out and thoroughly mixed by hand, in order to insure more even distribution of the virus. The pan and contents were then weighed and later brought up to the original weight at regular intervals by the addition of water.

The dry plant tissues used for soil inoculum were merely added to the moist soils in the proportion of about 1 part to 25 parts of soil, and allowed to remain there until sampled. The infested soils were placed in storage according to requirements as to environment and tested every week, fortnight, or month for virus concentration. Various modifications of this general plan of experimentation were introduced according to circumstances, and these will be briefly discussed in connection with individual experiments.

The presence and relative concentrations of tobacco mosaic virus in any lot of soil was determined by means of the local-lesion method, inoculations being made into *Nicotiana glutinosa* or into the hybrid, *N. glutinosa* × *N. tabacum* (*5*). For the type of investigation planned, this method offers many advantages over the older method of inoculation to *N. tabacum*. Use of the hybrid in place of *N. glutinosa* was also found to increase the range and reliability of the determinations and was adopted for most of the experiments.

Tests of the concentration of virus present in a soil or tissue therefore consisted simply of extracting a given volume or weight of the material with water and rubbing the extract over the leaves of young hybrid or *Nicotiana glutinosa* plants. Samples of soil for inoculation were usually taken by inserting a sterilized ⅜-inch cork borer into the soil to a depth of 1 inch and ejecting the sample by means of a sterile glass plunger. The sample was placed in a sterilized test tube and 5 cc of water added. After two vigorous shakings, the soil was allowed to settle out for from 2 to 5 minutes and the supernatant liquid poured off into a watch glass and inoculated into the test plants. The average dry weight of soil samples taken by this method was approximately 1 g. When plant parts were used for soil inoculum, the samples were taken from the tissues themselves, or what remained of them following decay. These were necessarily estimated by volume, about 2 to 5 g, according to the tissue, being taken for extraction with 10 cc of water. Original extract used for control inoculations was usually stored at a dilution of 1 to 1 or 1 to 20, but was brought to a final dilution of 1 to 1,000 of the original strength just before inoculation.

Counts of the number of local lesions on the test plants could usually be made on the third or fourth day after inoculation. In the case of the hybrid, these were based on the total number of spots

appearing on the three leaves showing the maximum amount of infection (usually four or five leaves being inoculated on each plant), and in the case of *Nicotiana glutinosa*, on a similar five-leaf count. Lesions numbering less than about 300 were usually counted accurately, while those above 300 were sometimes only estimated, and are then shown in round numbers in the tables. While the plants used in any one series of tests could be selected for uniformity of size and vigor, it was not always possible to secure comparable plants for successive tests made on the same material throughout the year. This factor, together with the natural individual differences in the test plants, introduced considerable variation into the determinations. This was counteracted, in part, by making counts on selected leaves, and in certain cases by using two test plants for each determination and taking the average of the two readings. It is not believed that the method of extraction of the virus from the soil was responsible for much variation in results. The number of shakings, or the time allowed for the soil to settle did not appear greatly to influence the number of lesions obtained. That all the virus was not, of course, removed from the soil by this method could be shown by repeated extractions from the same sample. For the type of information sought in the present investigation, however, namely, the presence or absence of virus or a rough approximation of its concentration, the experimental methods used were considered satisfactory. For more detailed studies, these methods could no doubt readily be modified.

EXPERIMENTAL RESULTS

The data presented in the following tables for the most part illustrate representative tests on the various factors under consideration. Slight modifications introduced into successive trials of the same general nature make it impractical to present all the data on which conclusions are based.

INFLUENCE OF VARIOUS FACTORS ON THE BEHAVIOR OF THE TOBACCO MOSAIC VIRUS IN THE SOIL

Leaching From Mosaic-Infected Tissues

In planning experiments on the behavior of the tobacco mosaic virus in the soil, it may be assumed that the virus characteristically overwinters in the dead tissues of preceding crops remaining in the field after harvest. It is possible that the primary infection occurring on succeeding crops results entirely from a direct transfer of virus from such plant parts to the new crop. On the other hand, it has been the writers' experience that the tobacco mosaic virus is quickly and easily extracted from dead tissues by water in contact with such material, and it seems likely that a similar condition occurs in the soil itself. Consequently, it may be concluded that soil in the immediate vicinity of virus-carrying tissues contains virus that has leached out from the plant parts during periods of rainfall. How far the virus may leach out and how far it may be carried in soil water must depend upon a number of circumstances. Some simple preliminary experiments have shown that a large part, if not all, of the virus present in dead tissues may eventually be leached out and that it may be transported in this process for considerable distances, and by surface drainage presumably almost indefinitely.

In one experiment, 25 g of Connecticut soil was placed in each of six stoppered Gooch funnels to a depth of about 1 inch. The soil was soaked down with water, and dried infected roots, stubble wood, stalks, sucker stems, and leaf lamina, and living green leaves were placed in separate funnels on the surface of the soil. The plant parts were moistened, and the corked funnels allowed to stand in a warm greenhouse (about 85° F.) to favor rapid decomposition of the tissues. At weekly intervals, 5 cc of water was added to the plant parts and allowed to drain through the soil and out of the funnel, where it was collected and inoculated into hybrid plants. The results presented in table 1 show that considerable quantities of virus were thus leached out from the tissues and were able to pass through the soil. When the soil filtrates no longer yielded infective material it was no longer possible to recover virus from the plant parts themselves by inoculation.

TABLE 1.—*Leaching of tobacco virus 1 through 1 inch of Connecticut soil (25 g) from various decaying tobacco plant tissues on the soil surface, following periodic addition of 5 cc of water*

Period of decomposition of tissues (weeks)	Lesions on 3 leaves of hybrid inoculated with soil leachings from—					
	Dried infected—					Green leaves
	Roots	Stubble wood	Stalks	Sucker stems	Leaf lamina	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
1	34	52	235	3	700	13
2	10	9	390	26	265	30
3	11	3	219	3	240	23
4	2	3	12	0	161	6
5	0	1	1	0	230	10
6	0	1	0	0	161	5
7					145	0
9					106	
11					[1] 7	
14					2	0

[1] Tissues accidentally dried out.

In another experiment, soil columns 18 inches in height were used in cylindrical copper containers, and virus, added in the form of extract at a dilution of 1 to 100, was found to filter for this distance through soils of various types, though much more readily through sandy than through clay soils. To eliminate the possibility that the virus had followed an uninterrupted path along the walls of the containing vessels, another test was made in which small collecting funnels were inserted into a soil column at different levels in such a way that the filtrate could enter the funnels only after passing directly through the soil for various distances. Inoculation of liquid collected from the funnels showed that virus had passed through the soil for all distances tested, in this case 2, 4, and 6 inches. It seems very likely that leaching of the virus from infected tissues in the field and the mixing of the soil due to preparation of the land for planting may result in a fairly uniform distribution of virus in the soil following heavily infected crops.

From these and other considerations it seems reasonable to assume that the area of soil contaminated through leaching is likely to be a more significant factor in soil infestation than the actual concentration of virus in the dead plant tissues. For this reason, as well as for purposes

of securing an even distribution of virus in the soils to be studied, infestation of soils was usually made with virus extract, though infected plant tissues were also used in several experiments.

Soil Type

Earlier tests by Johnson and Ogden (*6*) had indicated that the tobacco mosaic virus might remain active for a much longer time in some soils than in others. Aeration was suspected as being one factor influencing the longevity of the virus in the soil, and it was suggested that this might be related to the soil type. Consequently a preliminary experiment was planned, according to the methods already described, in which the behavior of the tobacco mosaic virus was studied in soils of different type. Seven representative soils were selected for the purpose (table 2) and placed in duplicate in 2-kg lots in the enamel pans. The soils were inoculated with 200 cc of virus extract diluted 1 to 1 with water, and as a control, 2,000 cc of virus extract at a dilution of 1 to 20 was also placed in each of two pans. One series of pans was allowed to stand in the greenhouse with the covers off to permit free aeration and drying of the soil, whereas the other series remained covered. Both sets were stirred and brought up to weight with water weekly, but the soil repeatedly became relatively dry in the uncovered pans in the intervals between weighings.

Soil samples were taken at various intervals and inoculated to both hybrid and *Nicotiana glutinosa* plants. The hybrid regularly yielded a much higher lesion count than did *N. glutinosa*, and as a result the hybrid was used as test plant in most subsequent experiments. It should be stated that in the present experiment the *N. glutinosa* plants used for inoculation purposes tended to be somewhat older than most suitable for this work, and this fact may be partly responsible for the low counts obtained, especially in some of the earlier inoculations.

Table 2.—*Relative rate of inactivation of tobacco virus 1 added in the form of extract to different moist soils stored at moderate temperatures in covered and uncovered pans*

[Pans brought up to original weight at intervals by addition of water]

Soil [1]	Pan cover	Average weekly loss of water	Lesions on test plant after indicated period							
			On hybrid			On *Nicotiana glutinosa*				
			0 week [2]	1 week	2 weeks	0 week [2]	1 week	4 weeks	7 weeks	12 weeks
		Grams	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Wisconsin (B)	On	6	600	600	500	250	65	42	70	68
	Off	93	600	300	76	200	27	0	0	.....
South Carolina (2)	On	12	700	600	400	200	60	120	6	25
	Off	167	550	350	64	175	17	0	0	.....
Florida	On	8	500	500	500	200	44	83	7	18
	Off	170	350	200	300	225	3	0	0	.....
Ohio	On	4	450	125	135	85	6	12	3	15
	Off	166	350	102	42	49	1	0	0	.....
Pennsylvania	On	5	450	80	57	25	2	31	1	1
	Off	194	350	0	0	55	0	0	0	.....
Connecticut	On	8	275	315	300	94	14	21	12	14
	Off	163	250	60	8	45	6	1	0	.....
Tennessee (C)	On	5	97	36	36	2	1	6	2	5
	Off	149	200	0	1	27	0	0	0	.....
Virus extract (stored at 1 to 20 dilution)	On	14	275	600	500	175	18	0	0	.....
	Off	396	194	300	300	95	42	0	0	.....

[1] See explanation of letters and numerals on p. 272.
[2] Immediately after infestation with the virus.

Considerable variation will be noted in the number of lesions obtained in the duplicate inoculations from some of the soils made immediately after infestation with the virus (table 2). In spite of this variation, there is still some indication that the soils themselves may vary significantly in their immediate effect upon the virus. The reason for this is not clear, though at the time it was suspected that differences in adsorptive capacity of the soils might be partly responsible. On the other hand, it is possible that the differences noted might be due merely to the difficulty of securing perfectly uniform distribution of the virus in the soil. However, further evidence of a similar nature obtained in other experiments, to be discussed later, points to the existence of inherent differences in certain soils with respect to their immediate effect upon the virus.

Subsequent inoculations from the different soils (table 2) showed a gradual inactivation of the virus in the covered pans in the absence of drying and in reduced aeration. More striking was the relatively rapid and complete inactivation that occurred in the uncovered pans, where the soil was exposed to drying and more complete aeration. There was also some indication that the rate of inactivation was slower in certain soils, e. g. Wisconsin (B) soil, than in others, under the same conditions. Such differential behavior, however, will be more clearly demonstrated in later experiments discussed in connection with the aging or persistence of the virus in different soils, (see tables 17, 18, and 19). The preliminary experiment suggests that several different major factors need to be considered as playing a possible part in the inactivation of the tobacco mosaic virus under natural conditions, namely, adsorption, aeration, desiccation, and possibly microbial action. In subsequent experiments, attention has been given to these and other factors individually, while attempts have been made to maintain other conditions as constant as possible.

Adsorption

It is well known that charcoal and certain other substances of both organic and inorganic nature have a peculiar affinity for the tobacco mosaic virus, resulting in its partial or complete inactivation. This takes place presumably through adsorption of virus particles. It seems not unlikely that soils, varying greatly as they do in physical and chemical characters, might also exercise some adsorptive capacity for the virus. Indications that this might be so have already been noted in the marked variation obtained in initial lesion counts when virus extract was added to different soils (table 2). Accordingly, a more detailed comparison was made of the adsorptive capacity for the tobacco mosaic virus of 26 different soils, varying from light sands to heavy clays, with that of peat, charcoal, kaolin, kieselguhr, talcum powder, and glass powder.

Since it seemed likely that the adsorptive power of the soil would be influenced by its physical structure, more particularly by the percentage of colloidal material (clay) present, a physical analysis of the soils was made by the Bouyoucos hydrometer method (*1*). The percentage of total colloids, silt, and sand in the different soils is shown in table 3, together with the pH value of each soil as determined by the electrical method, and the results of three tests of the adsorptive capacity of different soils for the virus. In the first of these tests,

10 cc of concentrated virus extract was added to 100 g of air-dry soil and allowed to stand for 24 hours. Extracts of soil samples were then made in the usual way and inoculated into hybrid plants. In the second and third tests, 10 cc of virus extract at a 1 to 500 dilution was added to 10 g of soil, and allowed to stand for about 1 hour. Ten cubic centimeters of water was then added and the suspension filtered through filter paper. Still other tests were made in an attempt to compare the adsorptive capacity of certain soils with that of other substances of known high adsorptive power. Only selected results are presented in table 4, which shows a comparison of filtrates from New York and South Carolina (*1*) soils, sand, talcum powder, and animal charcoal, following the addition of equal amounts of virus extract to equal volumes of soil or other substances.

TABLE 3.—*Type, physical analysis, and hydrogen-ion concentration of the different soils used in the investigation, together with a comparison of the adsorptive capacity of different soils for tobacco virus 1 added in the form of extract*

Soil[1]	Type	Colloids	Silt	Sand	pH (electrical method)	Lesions on 3 leaves of hybrid from soil filtrate soon after virus was added in experiment no.—		
						1	2	3
		Pct.	*Pct.*	*Pct.*		*No.*	*No.*	*No.*
Wisconsin (R)	Superior red clay	50.4	13.2	36.4	5.4	275	135	.....
West Virginia	Huntington silt loam	48.6	29.0	22.4	5.5	150	80	200
Kentucky	Hagerstown clay (?)	44.4	33.0	22.6	7.4	105	.....	.....
Tennessee (Greenville) (C)	.....	39.0	24.4	36.6	8.1	82	180	535
Pennsylvania	Hagerstown loam	37.8	34.6	27.8	7.9	109	138	251
Tennessee (8)	Clarksville silt loam	36.2	43.2	20.6	7.1	304	156	390
Ohio	Miami clay loam	35.2	40.0	24.8	7.1	175	260	600
New York (Elmira)	.....	33.4	22.8	43.8	6.0	200	293	96
Wisconsin (W)	Carrington silt loam	31.8	34.2	34.0	5.8	.....	.....	176
Virginia	Cecil red clay (?)	30.2	24.6	45.2	5.0	375	.....	.....
Wisconsin (8)	Miami silt loam	29.8	43.2	27.0	8.2	162	112	.....
Greenhouse	Compost	28.0	26.4	45.6	7.5	98	.....	.....
Wisconsin (E)	Miami silt loam (?)	27.8	33.0	39.2	6.1	.....	.....	.....
Wisconsin (G)	Knox silt loam	20.8	44.2	35.0	4.8	.....	.....	.....
Massachusetts	Agawam sandy loam	17.8	19.0	63.2	5.5	150	162	.....
Wisconsin (C)	Waukesha silt loam	17.4	34.2	48.4	8.5	300	105	294
Connecticut	Merrimac sandy loam	14.6	15.6	69.8	5.5	665	106	259
Wisconsin (L)	Sparta sand	12.4	6.2	81.4	5.2	.....	.....	146
North Carolina	Durham sandy loam	12.0	5.2	82.8	6.1	325	120	119
Maryland	Collington fine sandy loam	9.4	7.8	82.8	5.7	.....	68	322
Florida	Orangeburg sandy loam	9.2	2.0	88.8	5.8	500	95	.....
Georgia	Norfolk sandy loam	9.0	7.0	84.0	5.3	350	.....	.....
South Carolina (3)	....do	8.0	2.4	89.6	7.1	625	.....	.....
Wisconsin (Brooklyn) (B)	.....	7.2	4.0	88.8	6.0	162	118	230
South Carolina (1)	Norfolk sandy loam	7.0	4.0	89.0	7.8	375	138	167
South Carolina (2)	....do	6.6	5.0	88.4	5.7	105	.....	347
Peat	Peat	?	?	?	7.1	375	.....	.....

[1] See explanation of letters and numerals on p. 272.

TABLE 4.—*Comparison of the adsorptive capacity of equal volumes of soils and other materials for tobacco virus 1, used in the form of extract diluted to 1 to 1,000*

Soil or material[1]	Lesions on 3 leaves of hybrid in experiment no.—		
	1	2	3
	Number	*Number*	*Number*
New York	293	347	107
South Carolina (1)	138	61	93
Sand	.....	110	90
Talcum powder	0	0	0
Animal charcoal	0	0	0
Virus extract only (1 to 1,000 dilution)	87	78	80

[1] See explanation of numeral on p. 272.

In these preliminary trials there were again indications of some immediate effect of the soil on the virus, though there appeared to be little correlation with the physical character of the soil, or with the hydrogen-ion concentration (table 3). On the other hand, none of the soils approached the high degree of inactivation exhibited by charcoal and other finely powdered substances, such as talcum, glass powder, kaolin, etc. During these and other trials, it became evident, however, that consistent and significant differences existed between certain soils with respect to their immediate effect upon the virus. This is more clearly illustrated in table 5, which shows a comparison of the immediate effect upon the virus of five selected soils when wet, air-dry, and oven-dry. In this test, 15 cc of 1 to 500 virus extract was added to about 10 g of soil, followed later by 15 cc of water, making a final dilution of virus of 1 to 1,000. The liquid was then filtered through filter paper and inoculated to hybrid plants. The difference between the New York and West Virginia soils is clearly significant, and apparently too great to be accounted for on the basis of differences in physical structure. On the other hand, it will be noted that the original moisture content of the soil had no significant effect on the amount of inactivation of the virus when added in the form of liquid extract.

It seems likely that some other, as yet undetermined, factor is involved in this rapid partial inactivation of the virus in the soil. For the present, it has not been thought necessary to investigate this particular phenomenon further. It will be shown later that this immediate partial inactivation bears no direct relation to the subsequent rate of inactivation of the virus following prolonged association with the soil. For practical purposes relating to mosaic control, it seems legitimate to regard all soils as essentially similar so far as immediate inactivation or possible adsorption of the virus is concerned. However, the physical properties of the soil may influence certain other factors, such as rate of dessication, which in turn may be indirectly related to adsorption phenomena; consequently adsorption may still be significant in relation to the ultimate rate of inactivation of virus in the soil.

TABLE 5.—*Comparison of amount of adsorption of tobacco virus 1 added in the form of extract to different soils of varying types and reactions and of different moisture content*

Soil [1]	Total colloids	pH	Lesions on 3 leaves of hybrid from virus added to—		
			Oven-dry soil	Air-dry soil	Wet soil
	Percent		*Number*	*Number*	*Number*
Wisconsin (C)	17.4	8.5	130	202	144
New York	33.4	6.0	225	184	181
Tennessee (C)	39.0	8.1	55	67	106
Wisconsin (R)	50.4	5.4	84	56	50
West Virginia	48.6	5.5	30	27	19
Virus extract only (1 to 1,000 dilution)				126	

[1] See explanation of letters on p. 272.

The untreated virus extract itself often produced fewer lesions on the hydrid than after addition to certain soils (tables 4 and 5). This situation has been observed repeatedly and is believed to be a consequence of one or both of two circumstances, namely, the reduction or breaking up of clumps of virus particles through shaking with the soil, or the result of the abrasive action of soil particles during the process of inoculation.

SOIL REACTION

Although the tobacco mosaic virus has recently been shown to be active in extract over a wide range of hydrogen-ion concentrations (*8*), it is conceivable that the reaction of the soil might have some influence on the immediate or subsequent inactivation of virus added thereto. The pH value of all soils used in this investigation was therefore determined by the electrical method (table 3) and checked by the Truog method, the latter giving slightly lower readings. The soils ranged from very acid (pH 4.8) to quite alkaline (pH 8.5). As shown in tables 3 and 5, there was no significant correlation, however, between the hydrogen-ion concentration of the soils and any immediate effect upon the virus. Moreover, although, as will be shown later, the subsequent rate of inactivation of the virus was found to vary considerably in certain soils, again there was no consistent relation between inactivation and the soil reaction.

A further test was made with two very acid soils obtained from western Wisconsin, in the vicinity of Gays Mills (Wisconsin (G) soil) and La Valle (Wisconsin (L) soil). Two kilograms of each soil was placed in each of four pans, and lime was added to each series in the proportion of 0, 10, 20, and 30 g per pan, respectively. The lime was thoroughly mixed into each lot of soil, and the lots were inoculated with equal amounts of virus extract. The soils were brought up to, and maintained at, the same moisture content by means of weekly weighings. Soil samples were taken at intervals over a period of 17 weeks and inoculated into *Nicotiana glutinosa* plants (table 6). Although the rate of inactivation of the virus appears to have been somewhat higher in the most acid lot of the La Valle soil, this was not so in the case of the Gays Mills soil, which was even more acid. Everything considered, it appears that the reaction of the soils did not exert any significant influence on the rate of inactivation.

TABLE 6.—*Rate of inactivation of tobacco virus 1 added in the form of extract to two different soils maintained at various hydrogen-ion concentrations by adding lime*

[Soil kept in a moist condition and stored at moderate temperatures]

Soil [1]	Amount of lime added per pan of soil	pH	Lesions on 5 leaves of *Nicotiana glutinosa* after—								Average number of lesions
			0 week [2]	1 week	3 weeks	5 weeks	8 weeks	10 weeks	15 weeks	17 weeks	
	Grams		*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	
Wisconsin (L)	0	4.89	40	52	79	103	17	46	8	6	44
	10	7.58	55	62	278	197	18	55	29	8	88
	20	8.25	131	71	184	151	10	40	34	10	79
	30	8.64	85	153	240	132	25	141	28	37	105
Wisconsin (G)	0	4.80	51	59	247	203	77	170	75	83	121
	10	7.43	22	43	147	101	43	39	21	15	54
	20	8.30	55	92	128	190	3	14	24	5	64
	30	8.33	139	71	286	183	10	70	9	13	98
Virus extract (1-1,000 dilution)			18	15	176	136	13	183	------	------	90

[1] See explanation of letters on p. 272. [2] See footnote 2, table 2.

Soil-Moisture Content

Perhaps the most variable factor to which soils are exposed is that of moisture content. It was early suspected that this factor would have some effect on the longevity of the tobacco mosaic virus in the soil through its influence on microbial activity. It is well known that this virus will persist for years in dried plant tissues, but that in the presence of moisture and air, which permit microbial action, the virus will gradually become inactivated (*6*). In the present connection, however, a quite different moisture relation is emphasized, namely, the immediate effect of dessication of the soil upon the virus.

This effect was first observed in a preliminary experiment designed to test the relation of the soil-moisture content to the rate of inactivation of virus added in the form of extract to the soil. Two widely different soils were chosen, one a South Carolina sandy loam (South Carolina (1)) and the other a black Wisconsin silt loam (Wisconsin (W)). Determinations were made of the water-holding capacity of each soil and 2-kg lots were then made up to, and maintained at, different moisture contents by addition of virus extract and water, as shown in table 7. The two lots planned as controls were kept in an air-dry condition and were consequently air-dried as soon as possible after addition of the virus extract. Inoculations made the following morning showed that the virus was already almost completely inactivated in both dried soils, although present in considerable quantity in all moist soils. Subsequent tests made over a period of 17 weeks showed a gradual decline in virus concentration in the moist soils and continued, almost complete inactivation in the air-dry lots. While there is some suggestion of variation in the rate of inactivation at different moisture contents apart from drying, within normal limits for plant growth, at least, this factor does not appear to be of major importance.

Table 7.—*Rate of inactivation of tobacco virus 1 added in the form of extract to two different soils stored at moderate temperatures and maintained at various moisture contents*

Soil [1]	Moisture content as percentage of saturation	Lesions on 5 leaves of *Nicotiana glutinosa* after—								Average number of lesions
		0 week [2]	1 week	3 weeks	5 weeks	8 weeks	10 weeks	15 weeks	17 weeks	
	Percent	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	
South Carolina (1)	100	137	333	196	104	163	175	87	33	153
	75	159	180	252	190	108	66	43	14	126
	50	153	328	284	160	205	58	30	14	154
	25	167	233	334	270	504	92	31	25	207
	[3] 0	5	14	7	24	5	7	4	0	8
Wisconsin (W)	100	88	36	80	291	70	14	2	5	73
	75	63	84	313	330	171	125	85	23	149
	50	66	130	91	310	193	147	66	21	128
	25	105	31	65	130	49	61	5	13	57
	[3] 0	1	0	0	1	0	1	0	0	0
Virus extract (at 1–1,000 dilution)		18	15	176	136	13	183	------	------	90

[1] See explanation of letter and numeral on p. 272. [2] See footnote 2, table 2. [3] Air-dry.

Various other experiments have been made to test this striking influence of drying on the inactivation of the virus in the soil. All have yielded results of a similar nature. Thus, exposure of red-clay soil (Wisconsin (R)) to drying for as short a time as 1 hour following

addition of virus extract reduced the virus content to at least one-fifteenth of the original amount, as judged by inoculations into hybrid plants. In sand the rate and percentage of reduction were least, whereas in other soils of intermediate type they were intermediate in this respect. This behavior suggests the possibility of some adsorption phenomenon coming into play during drying. It does not, however, eliminate the possibility that aeration plays some part in this rapid inactivation; consequently, further experiments were planned to test this relationship.

Aeration

The inactivating influence of oxygen on tobacco mosaic virus extract has previously been reported (*3*), and its relation to inactivation of the virus in the soil has been suggested (*6*). In soil studies, however, the marked effect of dessication on the virus necessitated the planning of more carefully conducted tests. Thus, various attempts have been made to separate the factors of aeration and dessication in different ways.

In one experiment, a soil rotator was devised, permitting constant stirring of the soil within a glass tube 4 inches in diameter, in which the amount of oxygen and atmospheric humidity could be approximately controlled by drawing air, conditioned as desired, through the rotator. Equally satisfactory results were obtained by placing smaller quantities (5 g) of soils in desiccators over either water or calcium chloride or sulphuric acid, and removing the oxygen when desired by means of pyrogallol potassium hydroxide or by displacement with illuminating gas (table 8). In a third experiment, 2-kg lots of soil were used in enamel pans either covered or uncovered, with daily regulation of soil moisture and stirring of the soil, where desired, over a period of 4 weeks (table 9). All methods yielded similar results. It became clear from these trials that, while the presence of oxygen may cause a definite increase in the rate of inactivation of virus extract in the soil over considerable periods, it can in no way be held responsible for the immediate or rapid inactivation of the virus resulting from exposure of the soil to air-drying. It may be concluded that it is the actual desiccation of the soil that causes the rapid inactivation of virus extract present therein. It must not be inferred, however, that a similar behavior results on the part of virus present in organized plant tissues in desiccated soils. This situation will be discussed in detail later.

Table 8.—*Comparison of the influence of oxygen and of desiccation on the inactivation of tobacco virus 1 added in the form of extract to three different soils (desiccator method)*

Condition of atmosphere [1]		Regulation [2] by—	Lesions on 3 leaves of hybrid from soil from—		
Oxygen	Moisture		Connecticut [3]	Georgia	Virginia
			Number	*Number*	*Number*
+	+	Water	400	67	86
+	−	Calcium chloride	122	0	0
+	−	Sulphuric acid	51	0	0
−	+	Illuminating gas + water	400	213	60
−	+	Pyrogallic acid − potassium hydroxide + water	181	122	55
−	−	Illuminating gas + calcium chloride	38	0	0
Control (untreated infested moist soil)			660	75	91

[1] + present, − absent. [2] For 48 hours. [3] This soil was not completely desiccated.

TABLE 9.—*Comparison of the influence of aeration and drying, through regulation of air and water supply by periodic stirring and watering, on the inactivation of tobacco virus 1 in two soils in covered and uncovered pans*

[Virus added in the form of extract to 2-kg lots of soil]

Soil	Aeration	Water supply	Lesions on three leaves of hybrid after—			
			1 day	7 day	14 days	28 days
			Number	*Number*	*Number*	*Number*
Ohio	Low	High	440	74	124	372
	Medium	do	135	115	45	105
	High	Medium	154	163	46	18
	do	Low	372	105	18	1
North Carolina	Low	High	207	140	30	79
	Medium	do	221	150	12	10
	High	Medium	365	57	1	0
	do	Low	364	6	0	0

SOIL TEMPERATURE

Under natural conditions the soil is exposed to wide fluctuations in temperature, which might be expected to have some influence on the virus content of the soil. On account of the high thermal death point of the tobacco mosaic virus, however, little direct effect of high soil temperatures is to be expected. Such temperatures are likely to be concerned chiefly with the rate of microbial activity in the soil and the resultant decomposition of infected plant tissues. Judging from the behavior of the tobacco mosaic virus in extract at different temperatures, it might be anticipated that low soil temperatures would act in a preservative manner, preventing decay and generally prolonging the life of the virus. That this is not necessarily so will be shown in the following experiments, in which freezing temperatures in particular were found to cause relatively rapid inactivation of virus extract in the soil.

A preliminary experiment was planned to test the relative rate of inactivation of the virus in two soils (Wisconsin (S) and Maryland soils) at a series of temperatures ranging from approximately 0° to 40° C. (table 10). Virus was added to the soils in the form of extract and the soils were stored in a moist condition in 2-kg lots in covered pans, placed in incubators kept at constant temperature. Daily readings were made of the temperature of each incubator and were found to vary very little from week to week. The mean of the daily readings for each incubator is given in table 10. As a control, virus extract diluted to 1 to 20 with water was stored in small stoppered bottles, one of which was placed in each chamber.

At temperatures of approximately 5° to 30° C., significant differences in the rate of inactivation of the virus over a number of weeks were found to be either lacking or very slow to develop. At 40° C., inactivation was distinctly more rapid in both soils, though not so evident in the extract. The most striking effect, however, occurred at approximately 0° C., where the decline in virus content of both soils was even more marked than at 40° C., although the virus extract stored in bottles at that temperature showed no comparable change. It seems likely that the more rapid inactivation at higher temperatures was due to the rate or type of microbial activity. The inactivation at 0° C., however, was more obscure and necessitated additional tests at low temperatures.

TABLE 10.—*Influence of temperature on the rate of inactivation of tobacco virus 1 added in the form of extract to two different moist soils*

Extract in—	Mean temperature	Lesions on 3 leaves of hybrid after—										Average number of lesions
		0 weeks (9)	1 week	2 weeks	5 weeks	7 weeks	8 weeks	10 weeks	12 weeks	14 weeks	16 weeks	
	° C.	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	
Wisconsin (8) soil [2]	−0.4	406	85	47	28	42	9	16	4	.....	.....	80
	4.8	345	570	279	277	400	300	.....	.....	.....	.....	362
	8.3	722	703	406	351	399	560	156	58	154	161	367
	15.8	425	792	127	494	283	402	.....	.....	.....	.....	420
	19.6	.....	446	84	196	419	348	20	135	177	11	204
	24.2	543	354	174	84	150	201	.....	.....	.....	.....	251
	29.5	415	215	219	120	157	210	70	14	.....	.....	177
	40.3	540	423	10	25	3	34	8	8	.....	.....	131
Maryland soil	−2.0	366	192	63	76	116	50	7	10	.....	.....	110
	4.8	365	660	243	1, 250	606	444	.....	.....	.....	.....	595
	8.3	.....	703	275	866	712	630	710	830	520	590	648
	15.8	296	341	394	953	272	490	.....	.....	.....	.....	458
	19.6	550	407	169	780	826	593	664	670	441	398	550
	24.2	562	457	244	696	745	456	.....	.....	.....	.....	527
	29.6	554	340	307	634	560	440	248	326	100	115	362
	40.3	490	434	98	215	90	110	43	50	12	4	155
Water (stored at dilution of 1 to 20; tested at 1 to 1,000)	−0.4	295	53	188	363	170	311	258	247	.....	240	236
	4.8	.....	114	114	290	111	232	.....	.....	.....	.....	172
	8.3	.....	137	73	179	279	343	179	49	.....	30	159
	15.8	.....	314	86	171	320	168	.....	.....	.....	.....	212
	19.6	.....	308	110	256	92	285	365	158	.....	326	237
	24.2	.....	254	80	194	369	215	.....	.....	.....	.....	222
	29.6	.....	467	135	129	38	78	46	1	.....	1	112
	40.3	.....	72	58	172	64	194	223	202	.....	119	138

[1] See footnote 2, table 2.
[2] See explanation of letter on p. 272.

In a second and similar experiment the same two soils were tested at three different temperatures below freezing (approximately −1°, −10°, and −21° C.) in comparison with controls stored at approximately 8° C. (table 11). All soils were in a moist condition at the beginning of the experiment and were kept up to weight by the regular addition of water, where required. The freezing of the soil, regardless of the actual temperature reached, caused relatively rapid inactivation of the virus present therein, as compared with the controls. The frozen soils appeared to be in a very dry condition, although still maintaining their original weight. This was believed to be due to the low temperature causing the water to crystallize out of the soil in the form of ice. In subsequent experiments, therefore, various soils either partly or fully saturated with water were tested at temperaatures below and above freezing, with similar results (table 12). It is evident that freezing causes relatively rapid inactivation of virus present in the form of extract in the soil, presumably as a result of desiccation of the soil and not as a direct effect of the low temperatures, since there is little effect on the virus itself at these temperatures when tested either in extract alone (tables 10, 11) or in plant tissues in the soil (tables 14, 15). It seems certain, therefore, that, under field conditions, such virus as leaches into the soil prior to the freezing of the soil must become largely, if not entirely, inactivated. Hence it may reasonably be assumed that in cooler climates any free virus present in the soil at the time of field planting has leached into it during the spring, whereas in tropical or subtropical climates this particular form of inactivation must be normally lacking.

TABLE 11.—*Influence of freezing temperatures on the rate of inactivation of tobacco virus 1 added in the form of extract to two different moist soils*

Extract in—	Mean temperature	Lesions on 3 leaves of hybrid after—						Average number of lesions after first week
		0 week [1]	1 week	3 weeks	5 weeks	7 weeks	9 weeks	
	(° C.)	No.	No.	No.	No.	No.	No.	
Wisconsin (8) soil [2]	−1.4	201	64	22	7	8	.....	25
	−9.9	300	92	11	2	3	.....	27
	−20.6	402	15	24	2	0	.....	10
	+8.5	560	156	58	154	161	.....	132
Maryland soil	−1.4	456	340	265	25	27	4	132
	−9.9	444	380	126	78	3	3	118
	−20.6	490	870	224	106	1	5	241
	+8.5	630	710	830	520	590	98	550
Water (stored at dilution of 1 to 20; tested at 1 to 1,000)	−20.6	232	430	282	.....	260	91	266

[1] See footnote 2, table 2.
[2] See explanation of letter on p. 272.

TABLE 12.—*Influence of freezing temperatures on the rate of inactivation of tobacco virus 1 added in the form of extract to different soils either partially or fully saturated*

SOIL SEMISATURATED WITH WATER

Extract in—	Temperature		Lesions on 3 leaves of hybrid after—						Average number of lesions after first week
	Mean	Range	0 week [1]	1 week	2 weeks	4 weeks	6 weeks	8 weeks	
	(° C.)	(° C.)	No.	No.	No.	No.	No.	No.	
Pennsylvania soil	−1.7	−1.2 to −2.1	1,070	301	.....	390	9	7	177
	15.7	15.1 to 16.2	923	835	.....	465	50	106	364
Georgia soil	−3.2	−2.9 to −4.2	880	636	.....	256	52	17	240
	15.7	15.1 to 16.2	893	670	.....	730	350	560	577
Full strength extract (tested at dilution of 1 to 1,000)	4.8	4.3 to 5.6	.....	.....	.....	237	.....	302	269

SOIL SATURATED WITH WATER

Florida soil	−1.3	−0.9 to −1.7	200	.....	39	16	.....	.....	27
	[2]−4	7 to −19	135	.....	19	1	.....	.....	10
	16.0	15.8 to 16.4	310	.....	166	104	.....	.....	135
New York soil	−3.5	−3.1 to −4.3	193	.....	62	27	.....	.....	44
	[2]−4	7 to −19	72	.....	52	1	.....	.....	26
	16.0	15.8 to 16.4	55	.....	218	94	.....	.....	156

[1] See footnote 2, table 2.
[2] Soil outdoors (Feb. 4 to Mar. 4).

MICROBIAL ACTION

The extreme longevity of the tobacco mosaic virus in bottled liquid extract, without preservatives, and in dry plant tissues is an outstanding characteristic of this virus. The fact that the addition of preservatives prolongs the life of the virus in extract suggests that such slow inactivation as occurs under these conditions may be due partly to decomposition of plant materials through microbial activity, which may be expected to be retarded by the limited oxygen supply and by the toxic effect of the products of decay. Inactivation of the virus is more rapid in moist plant tissues than in bottled extract, perhaps on account of a more plentiful supply of oxygen favorable for microbial growth and also of the supply of cellulose and other sub-

stances available as a medium for microbial, particularly fungous, development. It has repeatedly been shown in this and other investigations that virus extract becomes inactivated only very gradually in the soil at moderate temperatures, and, as indicated in an earlier paper (*6*), the virus is inactivated even more slowly, if at all, when soil and virus are brought together under aseptic conditions. This suggests that decomposition by itself may result in inactivation.

Although it is believed that much of the virus naturally present in the soil exists in the form of extract leached from infected plant tissues, the writers' experiments indicate that virus present in the soil in this form is subject to more vigorous types of inactivation than that remaining in plant tissues. Thus, tests of the persistence of virus in infected plant parts incorporated into the soil have shown that freezing does not cause inactivation of the virus present in the tissues (tables 14 and 15), nor does desiccation of undecayed tissues (table 14). According to the writers' observations, the gradual loss of virus from dead plant parts below the soil surface, under natural conditions, must result largely either from leaching out of the virus or from decomposition, or, more probably, from a combination of both factors.

The rate of decomposition of the tissues and of inactivation of the virus probably depends on a number of factors, such as the size and type of the plant parts (roots, stalks, leaves, etc.) and on the soil temperature, moisture, and air supply, as well as on the soil character and types of organisms present. In the present investigation, attempts have been made to compare the extent and rate of such decomposition under different conditions by exposing various types of virus-infected tissue, such as roots, stalks, and leaves to decay at different temperatures in a very moist atmosphere in the absence of soil (table 13), and under winter conditions in the presence of soil (tables 14 and 15).

In the first experiment, dried roots, stubble wood, stalks, and leaf lamina, fresh green leaves, and virus extract were placed in separate glass beakers set on a layer of moist sand in each of five enamel pans. Four pans were kept closed, the fifth open (table 13). Both plant parts and sand were kept very moist by frequent addition of water. The pans were placed under different temperature conditions, as shown in table 13, and inoculations were made at intervals by the usual method from samples of the different materials. As might be expected, decay of the tissues proceeded at a much higher rate at the higher temperatures than at the lower, and the rate of inactivation of the virus in the tissues appeared to be correlated with the rate of decomposition. Thus, at a temperature of 2° to 3° C., the virus content of the different plant parts remained very high, even after 20 weeks, while at the highest temperature, about 39° to 41° C., at which decay was rapid, the virus content diminished rapidly. It was thought that virus present in the woody tissue of the large roots and base of the stalk might survive longer than that present in the more succulent leaf or sucker tissue. It was found difficult, however, to obtain strictly comparable data on this point, owing either to wide differences in concentration of the virus present in the tissues or to difficulties of extraction. Nevertheless, the results tend to indicate that the stubble and roots of the crop are no more likely to preserve the virus, by virtue of their size and composition, than is the leaf tissue under the same environment.

TABLE 13.—*Rate of inactivation of tobacco virus 1 in various tobacco plant tissues allowed to decay naturally at different temperatures in the absence of soil*

Temperature range (° C.) and how kept	Number of lesions on 3 leaves of hybrid after indicated period resulting from virus from—									
	Roots									
	0 [1]	2 weeks	3 weeks	4 weeks	7 weeks	9 weeks	12 weeks	16 weeks	20 weeks	24 weeks
2 to 3 (refrigerator)	82	...	146	122	370	88	360	170	390	76
−30 to 18 (outdoors)	...	75	23	88	54	13	47	94	0	2
16 to 27 (indoors; pan closed)	...	202	20	8	14	25	9	...	...	...
16 to 27 (indoors; pan open)	...	...	36	7	0	1	...	...	...	...
39 to 41 (incubator)	...	7	0	0	1	0	...	...	...	...
	Dried leaf lamina									
	0 [1]	2 weeks	3 weeks	4 weeks	7 weeks	9 weeks	12 weeks	16 weeks	20 weeks	24 weeks
2 to 3 (refrigerator)	157	...	27	275	11	227	650	250	800	550
−30 to 18 (outdoors)	...	36	185	75	68	126	45	85	400	600
16 to 27 (indoors; pan closed)	...	420	850	360	150	18	82	...	13	21
16 to 27 (indoors; pan open)	...	...	76	75	180	189	186	...	...	...
39 to 41 (incubator)	...	258	76	34	3	25	...	...	...	...
	Stubble wood									
2 to 3 (refrigerator)	41	...	58	74	293	161	103	180	420	240
−30 to 18 (outdoors)	...	21	3	0	12	3	26	38	...	0
16 to 27 (indoors; pan closed)	...	252	3	133	68	21	...	...	...	...
16 to 27 (indoors; pan open)	...	...	117	34	64	4	...	...	...	...
39 to 41 (incubator)	...	92	6	2	0	0	...	...	...	...
	Fresh green leaves									
2 to 3 (refrigerator)	765	...	725	580	194	51	425	325	310	600
−30 to 18 (outdoors)	...	480	700	800	64	9	70	171	235	132
16 to 27 (indoors; pan closed)	...	192	92	118	235	64	37	...	...	...
16 to 27 (indoors; pan open)	...	...	102	206	51	16	0	...	...	...
39 to 41 (incubator)	...	209	234	320	66	3	...	...	...	...
	Stalks									
2 to 3 (refrigerator)	53	...	3	93	123	152	574	230	440	400
−30 to 18 (outdoors)	...	120	13	45	28	4	59	65	4	...
16 to 27 (indoors; pan closed)	...	260	51	21	1	21	...	...	...	...
16 to 27 (indoors; pan open)	...	...	22	6	2	0	0	...	...	...
39 to 41 (incubator)	...	3	1	6	0	0	...	...	...	...
	Extract (stored at 1 to 10 dilution)									
2 to 3 (refrigerator)	387	...	258	71	85	75	210	96	...	[2] 135
−30 to 18 (outdoors)	...	290	103	60	143	84	136	144	[2] 192	
16 to 27 (indoors; pan closed)	...	296	65	84	108	2	5	...	[2] 10	[2] 36
16 to 27 (indoors; pan open)	...	...	21	8	0	0	0	...	...	...
39 to 41 (incubator)	...	0	7	0	0	6	...	...	...	...

[1] Immediately after infection with the virus. [2] Virus not diluted further for inoculation.

TABLE 14.—*Rate of inactivation of tobacco virus 1 in various tobacco plant tissues placed in 2 moist soils and exposed to winter conditions in Wisconsin*

[Wisconsin (S) soil (pH 8.2) and Wisconsin (L) soil (pH 5.2) used;[1] experiment begun Sept. 27, 1934]

Date	Minimum temperature over preceding period	Lesions on 5 leaves of *Nicotiana glutinosa* from—											
		Plant parts in 2 different moist soils [2]						Plant parts stored dry in warm room without soil					Extract (stored at 1 to 1 dilution, tested at 1 to 1,000)
		Roots	Stubble wood	Stalks	Sucker stems	Leaf lamina	Extract	Roots	Stubble wood	Stalks	Sucker stems	Leaf lamina	
1934	°F.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	
Sept. 27						46	50	54	35	64	3		
Oct. 4	37	74	50	92	39	98	111	221	485	16	148	635	170
Oct. 11	45	136	67	376	50	60	78	687	744	199	820	1,270	69
Nov. 1	28	7	9	14	16	98	64						
Nov. 8	27	35	78	151	4	325	170	265	508	350	520	900	180
Nov. 22	26	174	131	111	106	178	54						270
Dec. 19	4	17	6	20	4	177	2	246	312	54	285	554	67
1935													
Jan. 16	−8	16	2	5	3	58	0						
Feb. 14 [3]	−22	79	2	20	6	130	1						
Mar. 14 [3]	−3	11	3	76	8	151	1						
Apr. 9 [3]	19	88	15	71	39	84	0						
May 9 [3]	21	8	2	5	2	140	0						
June 9 [3]	38	41	1	7	9	46	0	46	48	16	156	1,250	
Total		686	366	948	286	1,591	531	1,519	2,132	699	1,932	4,609	756
Average		57	30	79	24	122	41	253	355	116	322	922	151

[1] See explanation of letters on p. 272.
[2] Figures represent average of readings from 2 different soils.
[3] Inoculations made to 3 leaves of hybrid plants.

TABLE 15.—*Rate of inactivation of tobacco virus 1 in various tobacco plant tissues placed in 2 moist soils and exposed to winter conditions in Wisconsin*

[Wisconsin (S) soil (pH 8.2) and Wisconsin (L) soil (pH 5.2) used;[1] experiment begun Dec. 4, 1934]

Date	Minimum temperature over preceding period	Average number of lesions[2] on 3 leaves of hybrid inoculated from—						Total number of lesions
		Roots	Stubble wood	Stalks	Sucker stems	Leaf lamina	Green plants	
1934	°F.							
Dec. 4		190	37	38	270	1,200	900	2,635
1935								
Mar. 5	−22	136	88	66	37	553	368	1,248
Apr. 3	13	47	38	50	23	475	380	1,013
May 2	21	27	17	15	5	177	147	388
June 2	30	36	52	3	11	200	363	665
June 20	43	12	18	5	18	175	462	690

[1] See explanation of letters on p. 272.
[2] Average of readings from 2 different soils.

In a second experiment, the various plant parts were added separately to two different moist soils (Wisconsin (S) and Wisconsin (L) soils) in 6-kg lots placed in open wooden boxes set outdoors in early autumn in a shelter and covered with heavy canvas; hence, they were exposed to considerable microbial action before winter temperatures set in. The soils were kept moist by regular addition of water. Inoculations were made at intervals from the plant parts and from similar dried tissues kept as stock material (table 14). The volume of roots, stubble wood, stalks, and sucker stems taken as samples for extraction with 10 cc of water could only be roughly estimated, so that the relative value of the lesion counts obtained is less than in trials where measured quantities of soils were taken. Samples from soils infested with dried leaf lamina or with virus extract were taken by the usual cork-borer method.

The results are significant chiefly in showing the period the virus may persist in plant parts exposed to decay under more or less natural over-wintering conditions, and at the same time showing that a gradual reduction of virus content occurs in the tissues. Virus was recovered from the plant parts in the soil as long as 8 months after the beginning of the experiment. At the end of this period, the tissues were in a partly decayed condition, though not so markedly so as in the preceding experiment conducted in the absence of soil. Freezing of the soil did not cause any rapid inactivation of virus present in the plant tissues.

A third experiment, conducted along similar lines with the same soils but started later in the season (Dec. 4), at about the time of the first killing freeze, yielded similar results (table 15). It appears from these tests that the virus present in plant tissues below the soil surface is as resistant to inactivation as the tissue is to decay, i.e., some virus may remain in the tissues in moist soil until they are completely disorganized by decay, provided, of course, that excessive leaching out does not occur in the meantime. This was avoided in the present experiments since the soil lots were protected from direct rainfall. No significant differences were observed in the effect of decay upon the rate of inactivation of the virus in the respective plant parts.

Preliminary tests have shown, however, that when virus-carrying plant tissues in advanced stages of decay are exposed to air-drying, the virus may be rapidly inactivated (table 16), presumably as a result of drying, as in the case of virus extract in desiccated soil. The problem of weathering of infected plant tissues on or above the soil surface may consequently be quite different from that dealing with the persistence of the virus in tissues overwintering beneath the soil surface.

TABLE 16.—*Influence of air-drying on rate of inactivation of tobacco virus 1 in badly decayed plant tissues*

[Tissues exposed to decay for 24 weeks at 2°–3° C. before air-drying]

Tissue	Lesions on 3 leaves of hybrid inoculated from—		Tissue	Lesions on 3 leaves of hybrid inoculated from—	
	Tissues air-dried for 8 days	Tissues not air-dried (control)		Tissues air-dried for 8 days	Tissues not air-dried (control)
	Number	*Number*		*Number*	*Number*
Roots	2	76	Cured leaf	83	550
Stubble wood	0	240	Green leaf	11	600
Stalks	0	400			

SURVIVAL TIME

Evidence has previously been presented (*6*) that the tobacco mosaic virus may overwinter in the soil, that is, strictly speaking, may survive in the soil from the time of the first killing fall freeze to the time of planting in the spring. This period is naturally longest in the more northerly tobacco-growing districts. The maximum length of this period is usually not more than 8 months and may sometimes be considerably less in the North, while in southern tobacco-growing districts it may average less than 2 months.

A number of experiments have been conducted to test the persistence of the tobacco mosaic virus in different soils under various conditions. Experiments have already been described showing the persistence of the virus for at least 8 months in different plant tissues in the soil when exposed to natural winter conditions in Wisconsin (tables 14, 15). The results presented in table 14 show that the virus is able to survive the overwintering period in small portions of infected tobacco plants and that considerable active virus is still present in the decaying tissues at planting time the following spring. The data suggest that the roots and stubble of the plant are not more conducive to overwintering of the virus than is leaf tissue under the same environmental conditions. In the field, leaf tissue may in some instances be a particularly common form of virus-carrying refuse from both first and second growth. Consequently, it may follow that the actual amount of virus overwintering in plant tissues will depend greatly on certain cultural practices, such as time of plowing, and upon other environmental factors, e.g. precipitation, to which infected plant tissues are exposed. However, it is not proposed to discuss these more practical aspects in detail at present.

Other tests of the persistence of virus in the soil have been made, using inoculum in the form of extract, which permitted a fairly uniform distribution of the virus throughout the soil.

In one experiment, virus was added as extract in the usual manner to 2-kg-lots of moist soil in covered pans. Ten different soils were used, as shown in table 17. The pans were placed in a shelter outdoors in the early fall (Sept. 26) for exposure to natural winter conditions, and samples for inoculation were taken at intervals until the middle of the following February. The results presented in table 17 are indicative of the general trend of subsequent trials in that no significant differences were apparent between most of the soils tested, though a few seemed to be definitely less favorable to the survival of the virus in the soil. The virus content of most soils remained at a high level for about 2 months and then dropped suddenly in all soils during December. This is believed to be a result of freezing of the soil (tables 10, 11, and 12), since the sudden decline was correlated with a marked drop in outdoor temperatures below freezing, as indicated in table 17. The experiment was then repeated with soils stored at room temperatures and not exposed to either freezing or drying. Ten of the more outstanding soils were selected on the basis of their behavior in previous trials, and these are listed in table 18. This table shows that inactivation proceeded considerably more slowly in the West Virginia and New York soils, for example, than in the Wisconsin (C) and Pennsylvania soils, but that it was slow in most soils as compared with pure sand. Similar results have been reported in earlier comparisons of sand and soil (*6*), but the theory previously offered that this phenomenon is related to aeration seems no longer tenable.

TABLE 17.—*Persistence of tobacco virus 1 added in the form of extract to different moist soils exposed to winter conditions in Wisconsin*

Date	Minimum temperature over preceding period	Lesions on 3 leaves of hybrid inoculated from soil [1] from—									
		South Carolina (2)	Wisconsin (B)	Maryland	Wisconsin (E)	Connecticut	Wisconsin (W)	Tennessee (C)	West Virginia	Wisconsin (C)	Pennsylvania
1934	°F.	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Sept. 26		347	230	322	146	259	176	535	200	294	251
Oct. 3	37	537	827	875	530	610	354	239	426	593	254
Oct. 17	44	900	850	1,000	720	441	588	172	174	284	202
Oct. 21	42	600	740	420	674	205	328	165	303	110	312
Nov. 13	26	367	357	307	239	503	168	32	157	32	75
Nov. 28	26	720	378	329	366	201	204	318	158	105	80
Dec. 18	4	62	4	35	12	9	15	0	14	2	1
1935											
Jan. 16	−8	1	0	1	2	1	2	1	0	0	0
Feb. 15	−22	0	0	0	1	0	2	5	0	0	0
Average		393	376	365	299	248	204	163	159	158	141

[1] See explanation of letters and numerals on p. 272.

TABLE 18.—*Persistence of tobacco virus 1 added in the form of extract to different soils stored at moderate temperatures and maintained in a moist condition*

Soil [1]	Lesions [2] on 3 leaves of hybrid after—						Average number of lesions
	0 week [3]	1 week	5 weeks	9 weeks	13 weeks	16 weeks	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	
West Virginia	328	81	350	178	155	322	236
New York	127	124	333	240	329	112	211
Ohio	583	194	223	17	15	70	184
Wisconsin (B)	191	169	196	94	180	123	159
Tennessee (S)	298	175	104	77	29	154	139
Connecticut	160	64	265	44	136	143	135
Pennsylvania	475	151	28	3	4	23	114
North Carolina	196	169	81	64	31	41	97
Sand	324	10	2	1	0	0	56
Wisconsin (C)	189	65	17	6	2	2	47
Virus extract (at 1 to 1,000 dilution)	44	31	35	40	65	.....	43

[1] See explanation of numerals on p. 272.
[2] Average of 2 separate readings.
[3] See footnote 2, table 2.

A further comparison of the West Virginia and Wisconsin (C) soils with sand is given in table 19. In this test, duplicate series were run at moderate temperatures, 4-months-old and fresh tobacco mosaic virus extract being used respectively. Again the rate of inactivation was lowest in the West Virginia soil and highest in sand, even when fresh virus extract was used, though in all cases the rate of inactivation was higher for the old extract than for the fresh. Similar tests made at the same time with lots of sand screened to four different particle sizes revealed no difference in the rate of inactivation of the virus in the different lots. The reason for this peculiar effect of sand on the virus is not understood.

Additional evidence of the ability of the tobacco mosaic virus in the form of extract to persist for long periods in the soil in the absence of drying and freezing is presented in table 20. In this experiment, virus extract was added to nine different soils stored at room temperatures and kept in a moist condition. The rate of inactivation was slow in all soils, and virus was still recovered from some soils as long as 12 months after the beginning of the experiment.

It was not unusual to find that the ordinary tobacco mosaic virus had become attenuated in varying degrees in soils in which the concentration of virus had become much reduced, that is, in soils yielding perhaps one to five local lesions upon inoculation to a hybrid plant. The presence of these attenuated forms could be demonstrated by inoculating soil samples into ordinary tobacco, which then developed systemic symptoms of varying degrees of mildness. Presumably, in this case, infection resulted from one or a few isolated virus particles, which had become attenuated in some way in the soil. That such attenuated forms represented strains of *tobacco virus 1* could be shown by inoculation back to the hybrid. It has previously been shown that similar attenuated strains can be obtained also from field soils (*6*).

TABLE 19.—*Comparison of the rate of inactivation of tobacco virus 1 added in the form of fresh and of 4-month-old extract to two moist soils of different type and to sand, and stored at moderate temperatures*

[Both old and fresh virus extract used in separate tests]

Soil	Total colloids	Virus extract	Lesions [1] on 3 leaves of hybrid after—								Total lesions
			0 week [2]	1 week	2 weeks	3 weeks	4 weeks	5 weeks	7 weeks	9 weeks	
	Percent		*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
West Virginia	48.6	Old	625	227	61	31	47	54	35	66	1,146
		Fresh	850	449	230	167	170	167	236	261	2,530
Wisconsin (C) [3]	17.4	Old	675	300	50	7	15	4	6	10	1,067
		Fresh	900	278	132	34	10	10	36	23	1,423
Sand	1.8	Old	525	42	6	1	4	0	0	1	579
		Fresh	538	157	45	35	69	20	42	7	913

[1] Average of 2 separate readings.
[2] Immediately after infestation with the virus.
[3] See explanation of letter on p. 272.

TABLE 20.—*Persistence of tobacco virus 1 added in the form of extract to different soils stored at temperatures varying between 50° and 100° F. and maintained in a moist condition*

Month	Lesions on 5 leaves of *Nicotiana glutinosa* inoculated from soil [1] from—								
	West Virginia	Florida	Georgia	Tennessee (C)	South Carolina (1)	Wisconsin (B)	Connecticut	Maryland	Wisconsin (8)
1934	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
June	175	600	350	125	350				
July [2]	60	45	80	8	175	600	275		
August [2]	56	50	15	18	12	70	12		
September [2]	30	45	23	2	21	68	14	550	543
October [2]			28	28	105	310	92	780	196
November	250	150	130	10	37	300	34	593	348
December	250	130	300	40	200	230	77	441	177
1935									
January	170	83	98	37	11	60	43	398	11
February	98	42	19	106	12	12	25	96	5
March	295	43	15	23	104	33	10	194	35
April	120	52	3	16	98	8	36	5	
May	38	[3] 0	[3] 0	12	[3] 0	20	28	152	36
June	140	0	0	25	0	76	25	62	162
Average	140	103	82	35	80	156	54	330	152

[1] See explanation of letters and numerals on p. 272.
[2] The low figures obtained for these months are due largely to the use of unsatisfactory *Nicotina glutinosa* plants for the tests.
[3] Soil accidentally dried out.

These experiments on the survival of the tobacco mosaic virus in the soil under laboratory and greenhouse conditions are believed to give sufficient evidence that the virus may persist regularly in various types of soils and under various environmental conditions for periods of a year or more. It is evident, however, that this survival is accompanied not only by inactivation of by far the greater portion of the virus originally present, but also by attenuation of virulence of a significant part of the remainder. While this high percentage of inactivation is very fortunate in connection with cultivation of the crop, it should be emphasized that after heavily infected crops the overwintering of only a small fraction (1 percent or less) of the virus

originally infesting the soil the previous autumn would yield sufficient inoculum to account for high percentages of infection on the crop the following year.

DISCUSSION

The greenhouse and laboratory experiments reported in this paper were not designed primarily in relation to the practical field aspects of the tobacco mosaic problem. It has previously been shown that the tobacco mosaic virus may survive the winter in the soil (*6*), but little information has been available on its behavior in the soil under the various conditions to which it is naturally exposed. Consequently it was felt that a preliminary study of the individual factors which might play a part in the inactivation or survival of the virus in the soil were fundamental to a further study of the disease under field conditions. The results obtained have yielded an unexpected amount of information in certain respects and should not only aid materially in an interpretation of the apparently inconsistent behavior of the disease in the field, but also in the planning and conduct of further experiments relating to field control.

Apart from field aspects, however, a number of observations have been made during the course of the experiments which may have some bearing on the nature of the virus itself. Such phenomena as the behavior of the virus in sand, its rapid inactivation in soil, in the form of extract, as a result of desiccation and freezing, and its apparent sensitivity to these same conditions in severely decayed tissues suggest starting points for new lines of investigation of a more fundamental nature.

SUMMARY

Greenhouse and laboratory experiments were conducted to determine the relative importance of various factors in the persistence or inactivation of the ordinary tobacco mosaic virus (*tobacco virus 1*) in the soil.

The virus was found to leach out readily into the soil from decaying infected plant tissues; consequently, the experiments were performed chiefly with virus extract or different plant tissues added directly to the soil. Representative soils obtained from the different tobacco districts of the United States were used in the investigation.

Addition of virus extract to certain soils resulted in immediate inactivation of an appreciable amount of the virus, but in no case did this approach the high degree of inactivation caused by charcoal or other highly adsorptive substances. Neither did this limited type of inactivation appear to be correlated with the physical character of the soil, in the presence of soil moisture. Desiccation of the soil, however, resulted in an immediate, and usually complete, inactivation of virus extract added thereto. The rate and degree of inactivation during drying was correlated to a considerable degree with the character of the soil. It is possible that inactivation due to drying is related to adsorption phenomena.

The degree of water saturation of the soil above a low minimum, and the range of hydrogen-ion concentration occurring naturally in soils, did not appear to affect the inactivation of the virus. Aeration evidently increased the rate of inactivation both directly and through its influence on microbial activity, but it operated relatively slowly.

Soil temperatures between 5° and 30° C. did not appreciably affect the rate of inactivation of virus extract in the soil. At 40° C., inactivation was definitely greater. Freezing the soil, however, caused rapid inactivation of virus extract in the soil, irrespective of the actual temperature reached, probably as a result of desiccation through freezing out of soil moisture. Neither freezing nor desiccation caused any appreciable inactivation of virus present in undecayed plant tissues in moist soil. Desiccation of severely decayed tissues, on the other hand, caused rapid inactivation of the virus remaining therein.

Exposure of moist infected plant tissues to decay in the presence or absence of soil resulted in a gradual loss of virus content, though considerable amounts of virus remained in the tissues until they became completely disorganized through microbial activity. The rate of this decay was naturally affected by temperature, moisture, and other conditions; nevertheless, considerable amounts of virus remained alive in the tissues after 8 months' exposure out of doors, when kept in soil and consequently not directly exposed to weathering. At temperatures above freezing, virus extract persisted in moist, poorly aerated soil for a year or more. The rate of inactivation was, however, definitely more rapid in some soils than in others, and was strikingly more rapid in pure sand than in field soils, for some reason not at present understood.

It is believed that the results presented should have considerable bearing on studies and observations made on the overwintering of the tobacco mosaic virus under field conditions, as well as on the planning of field experiments on the control of the disease.

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GROWTH, CHEMICAL COMPOSITION, AND EFFICIENCY OF NORMAL AND MOSAIC POTATO PLANTS IN THE FIELD [1]

By WINONA E. STONE

Assistant plant pathologist, Vermont Agricultural Experiment Station

INTRODUCTION

This paper presents a comparison of the growth, chemical composition, and efficiency of healthy and mosaic potato plants (*Solanum tuberosum* L.) grown in the field under the same conditions. In this investigation the daily efficiency of the lessened leaf spread of mosaic plants is paralleled with that of the much larger leaf spread of normal plants. The problem is: Is a unit leaf area of a mosaic potato plant as efficient per day as a unit leaf area of a normal plant? To determine this point two sets of data are necessary: (1) The daily size and increase in spread of foliage, and (2) the composition in green and dry weight, ash, carbon, starch, and sugar at the end of the measurements.

WORK OF OTHER INVESTIGATORS

The daily leaf spread had to be determined while the potato leaflets were attached to the plant. These measurements must be as accurate as possible if the results in yield, determined chemically, are to have any value or meaning. Darrow (*3*) [2] measured strawberry leaves by three methods—leaf product, leaf matching, and adjustable platform planimeter. Correlation between extent of leaf surface and length times breadth of all three leaflets was accompanied by only 0.2 percent error. Less accurate was the matching of leaves to patterns of known areas. The third method, which consisted of inserting the leaf under cellophane and measuring with a planimeter, could be used successfully on attached leaves, as could the first method. From the standpoint of ease of manipulation and accuracy, the leaf-product method appears to be the most successful. Gregory (*13*) took three linear and two angular measurements on *Cucumis* leaves and from these determined the area as for a hexagon. James (*14*), working with large numbers of plants, estimated areas by multiplying the average number of leaves by the average area of a well-chosen sample leaflet. Vyvyan (*24*), using the attached plants as negatives, made prints of bean leaves and determined the area of the prints. Eisele (*10*) obtained the area of corn leaves by multiplying the length by the breadth by 75 percent. A somewhat similar method was used in this work. Stone (*23*), from length and breadth measurements, reconstructed potato leaflets on a large scale. The areas of these diagrams were determined by means of a planimeter and reduced to natural size.

The chemical data on normal and mosaic plants, aside from those on yield at the end of the growth period, are fragmentary. Malhotra

[1] Received for publication Apr. 29, 1935; issued March, 1936.

[2] Reference is made by number (italic) to Literature Cited, p. 308.

(*16*) found little difference in the percentage of ash of normal and mosaic potato tops. Freiberg (*11*) confirmed these results with tobacco. Meyer (*17*) made the general statement that plants which store starch abundantly contain little soluble reducing and nonreducing carbohydrates in their leaves, while others which store no starch accumulate a high percentage of reducing sugars in their leaves. Dunlap (*7*, *8*, *9*) reported that mosaic reduced the percentage of reducing sugars, disaccharides, dextrins, starch, and pentosans. Brewer, Kendrick, and Gardner (*2*), Grandsire (*12*), Malhotra (*16*), and Schertz (*22*) found carbohydrates reduced in mosaic plants, or at least in the pale parts of diseased leaves. Dufrenoy (*6*) claimed that mosaic inhibited photosynthesis and reduced the sugar content of the leaves.

The practical importance of mosaic lies in the reduced yield of plants affected by it. Orton (*19*) found that diseased plants were smaller and the yield was 22 percent less than that of normal plants. Wortley (*25*) reported losses of 100 percent on 200 Triumph plants. Murphy (*18*) recorded a 58 percent loss in 682 Green Mountain hills. Rosa and Zuckerman (*21*) planted tuber units of rugose and mild mosaic plants for comparison with similar units of healthy plants. The rugose mosaic plants yielded 45.1 percent and the mild mosaic plants 57.6 percent as compared with the healthy plants.

METHODS

Green Mountain potato plants were started in the greenhouse. When of suitable size they were transplanted outdoors in a plot of heavy, wet clay soil that had been treated with ammonium sulphate and potassium nitrate. The plot was surrounded by a cheesecloth screen, 3 feet high, to exclude flea beetles and other insects. The plants were set 4 feet from the screen and 2 feet from each other in order to avoid shading. Whatever the effect, if any, of the cheesecloth, it was shared equally by all the plants. Any insects surmounting this barrier were removed by hand when the daily measurements were taken.

Preliminary plantings were made from the tubers finally selected as seed, to determine the condition of the seed, but since it is not possible to predict with certainty from one seed piece the condition of the other sprouts from the same tuber, the final plants differed in health from the trial plants. However, one normal strong plant remained in apparently healthy condition throughout the experiment, and four showing different degrees of mosaic were chosen for comparison. Each plant was produced by a one-eyed seed piece and only one tuber shoot was allowed to grow.

No attempt was made to name the mosaic according to accepted nomenclature. Two of the plants were slightly affected with mosaic and two were severely affected. Chemical data on the mosaic plants were combined and are presented in two groups, data on slight mosaic and on severe mosaic plants.

Measurements of the leaflets, made daily at 9 a. m., were begun on July 25, when the plants were 10 cm high, and continued until September 2 and 3. By that time the plants had reached the flowering stage and had completed their period of most active growth. Narrow strips of millimeter coordinate paper were used as measures. Three measurements were taken: Plant height, length and breadth of

each tip leaflet, and length and breadth of one of each pair of side leaflets. Leaflets under 7 mm in breadth and 14 mm in length could not be measured accurately. The potato has a compound leaf composed of tip leaflet and two to four paired side leaflets. Throughout this work the tip leaflet was identified as leaflet *a*, the side leaflets as *b*, *c*, and *d*, from the tip to the bottom of the leaf.

To convert the length and breadth measurements of the leaflets into areas, a table was compiled based on their relation to the area of a rectangle of the same dimensions. As a leaflet ages it changes from a long, slender lance shape to an oval with a broad base. For example, the actual area of a leaflet 9 by 17 mm was found to be about 75 cm^2 or 50 percent of 1.53 cm^2, the area of a rectangle 9 by 17 mm, while the area of an older leaflet 50 by 80 mm averaged 30 cm^2, or 75 percent of 40 cm^2, the rectangle area. A table was made up to include leaflets with all possible combinations of measurements. This table by actual trial was found to be as accurate as the method formerly employed by the author (*23*) and could be used much more quickly.

On September 2 and 3 the plants were removed from the plot. The green and dry weights of roots, tubers, stems, tip leaflets, measured side leaflets, and unmeasured side leaflets were determined.

In the following presentation of data the plant designated 1 was normal, plants 2 and 3 were slightly affected with mosaic, and plants 4 and 5 were severely affected. Only five plants were used in this experiment, for although the measuring was quickly done when the plants were small and the leaves few, as the leaves became more numerous the time required for making and recording measurements rapidly increased to 2 or 3 hours. At the height of growth, over 1,000 measurements were made daily. It would have been impossible to consider the data comparable had they been taken over a period of more than a few hours, which would necessarily have been the case had a larger number of plants been used.

The official chemical method of Munson and Walker (*1*) was used in making the sugar determinations. Starch was determined by the use of Taka-diastase, according to the method of Davis and Daish (*4*), adopted by McGinty (*15*). Ash and carbohydrate were the result of the ordinary combustion procedure.

GROWTH OF PLANTS

Figure 1 shows that the normal plant produced fewer leaves than any of the diseased plants, except plant 4, but the larger size of the normal leaves gave a greater actual surface exposed. The final area of the measured leaves of the normal plant was 2,000 cm^2, and that of the measured leaves of the mosaic plants averaged 920 $cm.^2$ The average area of the normal leaflets was 17.5 cm^2 and that of the mosaic, 7.3 $cm.^2$ Decrease in leaf area and leaflet size followed closely the increasing intensity of the disease.

Differences in growth of the plants in height did not appear to be significant (fig. 2) until stated in terms of percentage. The gradation from 650 percent total height increase in the normal plant to 148 percent in the most seriously affected plant was consistent with the observable severity of the disease.

The green weight of measured leaflets decreased with increasing severity of the disease (fig. 3). Green weight per square centimeter of leaf area varied, but the dry weight per square centimeter increased

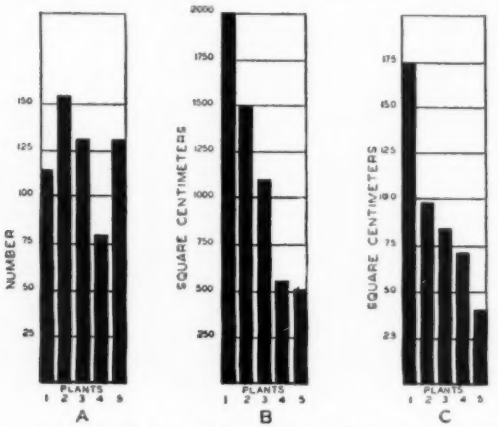

FIGURE 1.—Comparative final number (*A*), area (*B*), and average size (*C*) of all measured leaflets of plant 1, normal; plants 2 and 3, slight mosaic; plants 4 and 5, severe mosaic.

with increase in intensity of the disease. Possibly this is an indication of the more compact arrangement of cells in mosaic leaves which Dickson (*5*) found.

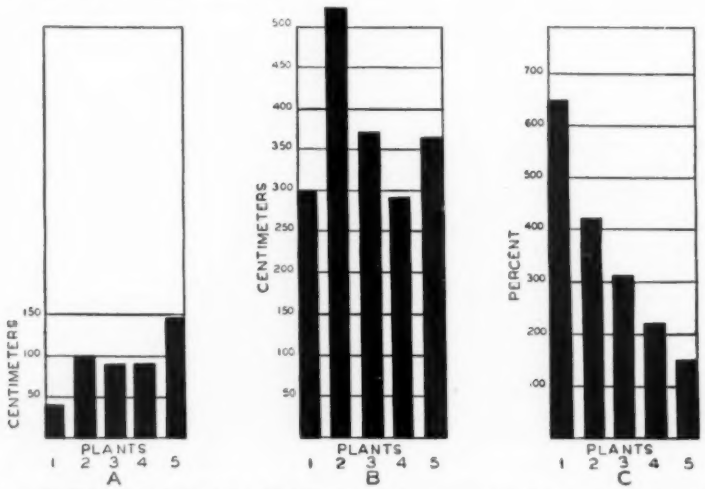

FIGURE 2.—Height at beginning (*A*) and end (*B*) of period of measurement, and comparative total percentage increase in height growth (*C*) of plant 1, normal; plants 2 and 3, slight mosaic; plants 4 and 5, severe mosaic.

Normal leaflets and leaves grew very rapidly for a short time then more slowly. Diseased leaves differed mostly in the amount of growth. They also usually showed an initial spurt, smaller to be sure,

and later they grew more slowly or not at all. Figures 4 and 5 show growth of comparable leaves and leaflets from normal and diseased

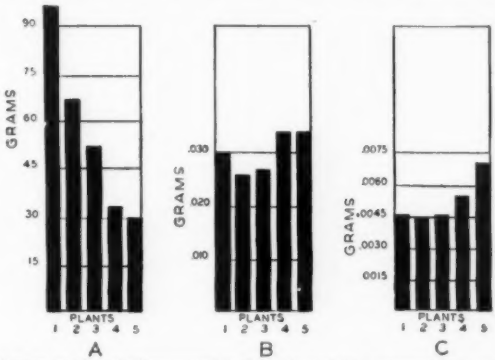

FIGURE 3.—Total green weights of all measured leaflets (*A*), and the comparative green (*B*) and dry (*C*) weights per unit of final leaf area of plant 1, normal; plants 2 and 3, slight mosaic; plants 4 and 5, severe mosaic.

plants. Figure 5 indicates that growth in young mosaic leaves was not so far below normal as in older ones, shown in figure 4. The

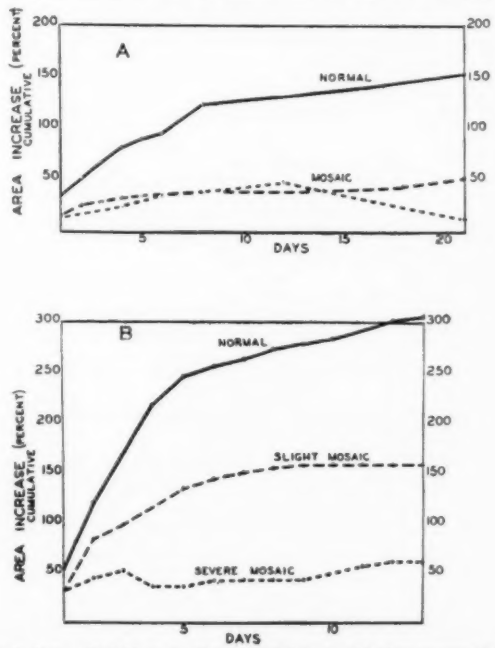

FIGURE 4.—Each day's growth of older normal and mosaic leaves and leaflets (percent) added to that of the previous day. Typical comparable normal and diseased leaves (13 in *A*) and tip leaflets (13 *A* in *B*).

growth of leaves was less rapid than that of their tip leaflets because of the much slower growth of the side leaflets. In 87 percent of all

the leaves measured the tip leaflet exceeded the side leaflets in growth, and in 56 percent of the cases leaflet *c* showed the least growth. The tip leaflet is a continuation of the rachis of this pinnately compound leaf. Growth appears to be greatest in the direction of the easiest flow of food material. Pearsall and Hanby (*20*) thus explained greater tip-lobe growth in ivy.

In apparent opposition to the foregoing is the fact that in the leaflets growth in breadth exceeded growth in length almost without exception.

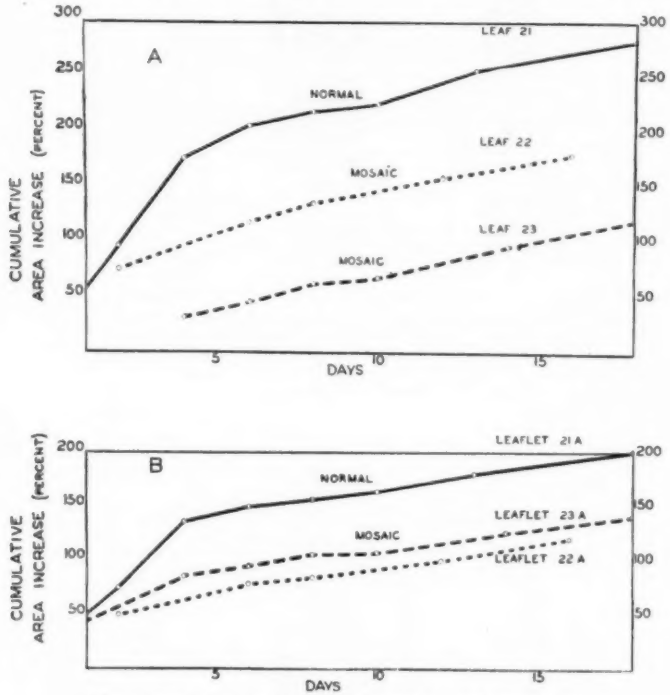

FIGURE 5.—Same as figure 4 but younger leaves in *A* and leaflets in *B*.

tion. The breadth-length growth relationship was more uniform in the normal plant.

In order to show the growth of the whole plant, the growth of every fifth leaf was plotted (figs. 6 and 7). In the normal plant (fig. 6) as growth fell off in one part of the plant it was picked up by a younger part. All normal leaves showed the typical rapid initial growth, but only the earlier mosaic leaves showed an initial spurt. Especially in the severely diseased plant (fig. 7, *B*) growth was without sequence, leaves 40 and 45 being larger and more rapid growers simultaneously with leaves 20 and 25, while leaves 30 and 35 exceeded 40 and 45.

Plant 2 (fig. 7, *A*), slightly affected with mosaic, was intermediate between the severely affected and the normal plants. The initial, accelerated growth was present in nearly all leaves but more subdued.

Growth passed on from leaf to leaf in an orderly manner. In plant 2 rather uniform and small sizes were attained by the leaves. When last measured, leaves of the normal plant were still actively growing and ranged widely in size.

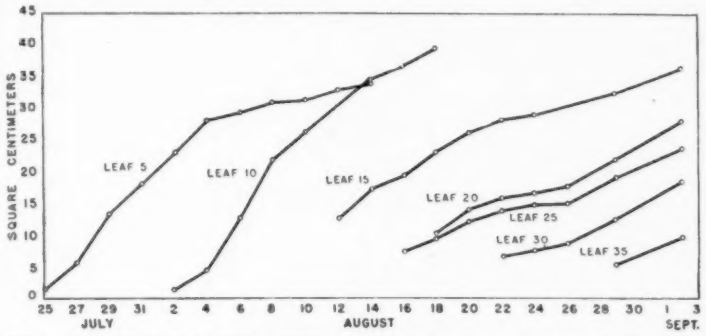

FIGURE 6.—Day-by-day area increase (cumulative) of a series of tip leaflets on the normal plant for the entire period of measurement.

Mosaic plants showed decreased daily leaf spread as well as smaller total leaf spread. The relative efficiency of the plants was determined by chemical analysis.

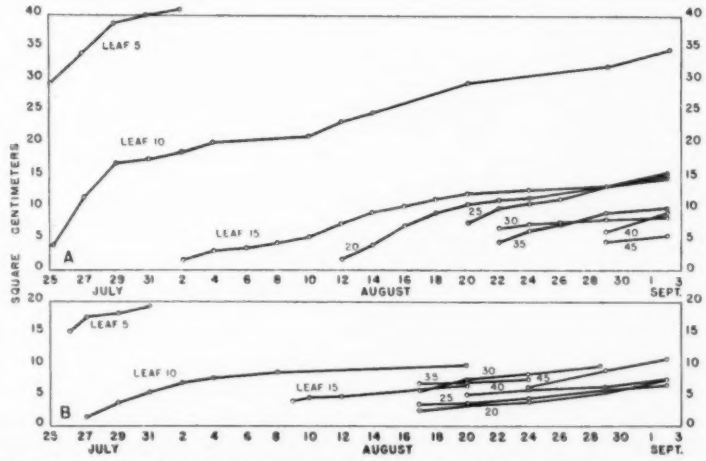

FIGURE 7.—Day-by-day area increase (cumulative) of a series of tip leaflets on a slightly mosaic-affected plant, no. 2 (*A*) and a severely mosaic-affected plant, no. 4 (*B*).

CHEMICAL ANALYSIS OF PLANTS

A summary of the results of the chemical analysis is presented in table 1. Data on the two slightly diseased plants (2 and 3) are combined, as are the data on severely diseased plants (4 and 5). The ash content was higher not only in the leaves of diseased plants, as has been reported by other investigators, but also in practically all other

organs and in the entire plant. Carbon varied little among the five plants analyzed. Sugar and starch content were quite generally higher in the normal plant.

TABLE 1.—*General results of chemical analyses of normal and mosaic potato plants*

Part of plant and condition	Green weight	Dry weight	Ash on dry basis	Carbon on dry basis	Sugar on dry basis	Starch on dry basis
	Grams	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Leaves:						
Normal	96.20	14.33	16.6	38.1	5.46	6.14
Slight mosaic	59.64	10.12	16.8	39.1	3.03	4.13
Severe mosaic	31.37	5.42	18.7	37.2	3.55	3.71
Stems:						
Normal	54.93	6.00	18.0	32.8	7.30	3.10
Slight mosaic	55.61	6.50	20.7	32.3	2.90	2.40
Severe mosaic	23.17	2.70	22.7	31.8	3.30	2.80
Tubers:						
Normal	172.00	33.25	4.6	38.8	6.10	66.20
Slight mosaic	54.85	9.00	6.8	38.2	3.80	53.50
Severe mosaic	35.50	6.35	5.0	38.5	6.50	50.60
Roots:						
Normal	6.50	1.30	21.2	33.3	(1)	(1)
Slight mosaic	7.91	1.67	24.2	32.7	(1)	(1)
Severe mosaic	3.40	.59	12.5	37.8	(1)	(1)
Plant (entire):						
Normal	329.60	54.90	9.6	37.8	6.10	43.00
Slight mosaic	178.00	27.30	14.8	36.8	3.30	21.10
Severe mosaic	93.40	15.10	13.4	36.6	4.70	24.00
Plant (exclusive of roots):						
Normal	323.10	53.60				
Slight mosaic	170.09	25.63				
Severe mosaic	90.00	14.51				

[1] Too small to determine.

Since the ash is made up largely of the elements absorbed by the roots, the relation of ash to root weight indicates the efficiency of the root. According to the data in table 2, the roots of the diseased plants were capable of absorbing about 75 percent as much per unit of weight as were healthy roots.

TABLE 2.—*Comparative efficiency of roots of normal and mosaic potato plants*

Condition	Dry weight of roots	Ash in plant	Ash per gram of root, dry weight
	Grams	*Grams*	*Grams*
Normal	1.30	5.26	4.0
Slight mosaic	1.67	4.06	2.4
Severe mosaic	.59	2.01	3.5

The proportion of carbon was fairly constant both among organs of the same plant and in those of normal and diseased plants, though the actual amount of carbon was much lower in the diseased plants.

The distribution of carbon among the organs paralleled more or less the distribution of dry weight (table 3). Mottling had a marked effect on the intake of carbon dioxide, though the degree of mottling appeared to be unimportant. The diseased plants accumulated about 17 percent less carbon than did the normal plant for each 100 cm^2 of leaf surface exposed each day (table 4).

TABLE 3.—*Amount and distribution of carbon in relation to dry weight of normal and mosaic potato plants*

Part of plant and condition	Carbon	Carbon on dry basis	Carbon as percentage of total carbon	Dry weight as percentage of total dry weight
	Grams	*Percent*	*Percent*	*Percent*
Leaves:				
Normal	5.46	38.1	26	26
Slight mosaic	3.96	39.1	39	37
Severe mosaic	2.02	37.2	36	36
Stems:				
Normal	1.97	32.8	10	11
Slight mosaic	2.10	32.3	21	24
Severe mosaic	.86	31.8	15	18
Tubers:				
Normal	12.90	38.8	62	61
Slight mosaic	3.44	38.2	34	33
Severe mosaic	2.44	38.5	45	42
Roots:				
Normal	.43	33.3	2	2
Slight mosaic	.55	32.7	6	6
Severe mosaic	.22	37.8	4	4
Plants (entire):				
Normal	20.76	37.8		
Slight mosaic	10.05	36.8		
Severe mosaic	5.54	36.6		

TABLE 4.—*Carbon in normal and mosaic potato plants in relation to leaf surface exposed per day*

Condition	Growing period	Total carbon	Carbon accumulated per day	Leaf surface exposed over growing period	Surface exposed per day	Carbon accumulated per 100 cm^2 of leaf surface per day
	Days	*Grams*	*Gram*	*Square centimeters*	*Square centimeters*	*Milligrams*
Normal	40	20.76	0.52	55,561	1,389	37.3
Slight mosaic	40.5	10.05	.25	33,307	822	30.4
Severe mosaic	37	5.54	.15	17,556	475	31.6

The leaf areas in tables 4 and 7 differ from those in figure 1, for the latter includes the area of only the leaflets actually measured. The presence or absence of the unmeasured twin was carefully noted for later determination of total leaf areas. The method of obtaining the total and daily area exposed over a 3-day period shown in table 5 was applied to the entire period of measurement and thereby the figures in table 4, columns 4 and 5, were obtained.

TABLE 5.—*Data indicating the method of calculating the total and daily leaf area exposed over a 3-day period for normal and mosaic potato plants*

Condition	Leaf area exposed on—			Total area exposed	Average daily area exposed
	Aug. 8	Aug. 9	Aug. 10		
	Square centimeters	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*
Normal	783.64	862.19	920.36	2,566.19	855.40
Slight mosaic	435.28	462.03	486.20	1,383.51	461.17
Severe mosaic	272.26	288.53	312.61	873.40	291.13

In comparing the daily efficiency of the leaves no account was taken of the fact that the length of day was less during the latter part of the experiment. Since all plants were grown under the same conditions of soil, moisture, temperature, and light, these external factors have not been considered in this investigation. So far as the individual plants were concerned these factors were constant, only the health of the plants varied.

The area of the severe mosaic leaves was actually greater than that recorded since it was impossible to follow the contortions of the wrinkled surface. Hence the severe mosaic leaves were less efficient than they are here pictured.

Diseased plants fell far below the normal plant in the quantity of food manufactured. From table 6 it may be seen that not only the leaves but also the stems and tubers, and indeed the whole plant, when diseased, manufactured and stored less starch and sugar, both in percentage and in weight. Here, again, the severity of the disease had little apparent effect on the proportion of sugar and starch in the plants.

TABLE 6.—*Sugar and starch content and distribution in normal and mosaic potato plants*

Part of plant and condition	Sugar	Starch	Sugar on dry basis	Starch on dry basis	Sugar as percentage of total sugar	Starch as percentage of total starch
Leaves:	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Normal	0.78	0.88	5.46	6.14	24.0	3.8
Slight mosaic	.31	.42	3.03	4.13	37.0	7.7
Severe mosaic	.19	.20	3.55	3.71	27.5	5.7
Stems:						
Normal	.44	.19	7.30	3.10	13.5	.8
Slight mosaic	.19	.16	2.90	2.40	22.6	2.9
Severe mosaic	.09	.08	3.30	2.80	13.0	2.3
Tubers:						
Normal	2.03	22.00	6.10	66.20	62.5	95.4
Slight mosaic	.34	4.82	3.80	53.50	40.5	89.4
Severe mosaic	.41	3.20	6.50	50.60	60.0	92.0
Plant (entire):						
Normal	3.25	23.07	6.10	43.00	----	----
Slight mosaic	.84	5.40	3.30	21.10	----	----
Severe mosaic	.69	3.48	4.80	24.00	----	----

Table 7 bears directly on the efficiency of a unit of leaf surface in relation to the storage of starch. The daily starch production by equal areas of foliage is markedly reduced in the mosaic plants. Since the sugar in the leaves on the final day represented for the most part the result of that day's photosynthesis, sugar content was determined on the basis of the final-day leaf exposure (table 8).

TABLE 7.—*Daily starch production per unit area of leaf exposed in normal and mosaic potato plants*

Condition	Total starch in—			Starch produced per day			Starch produced per 100 cm² of leaf per day		
	Tuber	Rest of plant	Entire plant	Tuber	Rest of plant	Entire plant	Tuber	Rest of plant	Entire plant
	Grams	*Grams*	*Grams*	*Gram*	*Gram*	*Gram*	*Milligrams*	*Milligrams*	*Milligrams*
Normal	22.00	1.07	23.07	0.55	0.03	0.58	39.6	2.1	41.7
Slight mosaic	4.82	.58	5.40	.12	.02	.14	14.6	2.4	17.0
Severe mosaic	3.20	.28	3.48	.09	.005	.095	18.9	1.1	20.0

Translocation, as well as actual manufacture, was slower and less complete in diseased plants. Figure 8 represents the percentage of the total starch and sugar content of the plant which remained in the tops at harvest. Mosaic-plant tubers received about 90 percent, whereas the normal tubers contained 95 percent. The diseased-plant tops retained an average of 51 percent of the total sugar of the plant as compared with only 37 percent retained by the normal tops. A

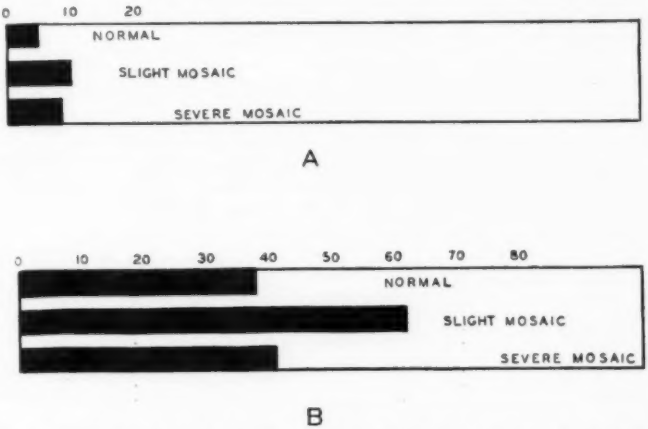

FIGURE 8.—Percentage of total starch (*A*) and sugar (*B*) remaining in the tops of normal and mosaic potato plants at time of harvest.

smaller proportion of starch in tubers accompanied a larger proportion of sugar in the tops (table 9). The fact that, in the diseased plants sugar made up about 15 percent of the sugar-starch content, but in the normal plant only 12 percent, further indicated that transfer of sugar and starch was interfered with by the disease.

TABLE 8.—*Sugar content of normal and mosaic potato plants per unit of final-day leaf exposure*

Condition	Total sugar in—			Final-day leaf exposure	Sugar per 100 cm² of leaf exposed		
	Leaves	Rest of plant	Entire plant		Leaves	Rest of plant	Entire plant
	Gram	*Grams*	*Grams*	*Sq. cm*	*Milligrams*	*Milligrams*	*Milligrams*
Normal	0.78	2.47	3.25	3,124	25	79	104
Slight mosaic	.31	.53	.84	1,983	16	26	42
Severe mosaic	.19	.50	.69	801	24	61	85

TABLE 9.—*Sugar in leaves in relation to starch in tubers of normal and mosaic potato plants*

Condition	Starch in tuber per gram of dry weight of leaves	Sugar in leaves per gram of starch in tubers
	Grams	*Gram*
Normal	1.54	0.035
Slight mosaic	.47	.064
Severe mosaic	.59	.060

The carbon-analysis data of tables 1, 3, and 4 included the carbon of sugar and starch but did not include the small amount of carbon in the ash, about 10 percent. More detailed data on carbon are presented in table 10. The percentage of total carbon which includes the carbon of the ash was even less variable than that of carbon as originally determined. However, the carbon of cellulose, lignin, etc., was quite consistently lower in the normal plant and organs. The normal plant kept more carbon in circulation in the form of sugar and available starch. The normal tubers showed a marked reduction in structural carbon.

TABLE 10.—*Carbon, including that in the ash, and carbon exclusive of that in the sugar and starch in normal and mosaic potato plants*

Part of plant and condition	Carbon in ash (about 10 percent)	Carbon as computed		Total carbon, 2 and 3		Carbon in sugar and starch (about 37 percent)	Carbon exclusive of that in sugar and starch, 5–7	
1	2	3	4	5	6	7	8	9
Leaves:	*Gram*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
Normal	0.24	5.46	38.1	5.70	39.8	0.61	5.09	35.5
Slight mosaic	.17	3.96	39.1	4.13	40.8	.27	3.86	38.1
Severe mosaic	.10	2.02	37.2	2.12	39.1	.14	1.98	36.5
Stems:								
Normal	.11	1.97	32.8	2.08	34.7	.23	1.85	30.8
Slight mosaic	.14	2.10	32.2	2.24	34.5	.13	2.11	32.4
Severe mosaic	.06	.86	31.8	.92	34.1	.06	.86	31.8
Tubers:								
Normal	.15	12.90	38.8	13.05	39.2	8.89	4.16	12.2
Slight mosaic	.06	3.44	38.2	3.50	38.9	1.91	1.59	17.7
Severe mosaic	.03	2.44	38.5	2.47	38.9	1.34	1.13	17.8
Roots:								
Normal	.03	.43	33.3	.46	35.4	0	.46	35.4
Slight mosaic	.04	.55	32.7	.59	35.4	0	.59	35.4
Severe mosaic	.01	.22	37.8	.23	39.0	0	.23	39.0
Plant (entire):								
Normal	.53	20.76	37.8	21.29	38.8	9.73	11.56	21.1
Slight mosaic	.41	10.05	36.8	10.46	38.3	2.31	8.15	29.8
Severe mosaic	.20	5.54	36.6	5.74	38.0	1.54	4.20	27.8

Yields in the present study must be considered significant in spite of the small number of plants involved. Yields from diseased plants were lower not only because of the decrease in the size of the tops, but also because the diseased tops were only about half as efficient in producing tubers as were the normal tops (table 11).

TABLE 11.—*Comparison of tuber weight in normal and mosaic potato plants*

Condition	Green weight	Ratio of tubers to tops, in grams
	Percent	
Normal	100.0	1.14:1
Slight mosaic	68.1	.48:1
Severe mosaic	79.4	.65:1

DISCUSSION

From the data presented it is clear that mosaic exerted a considerable influence on the growth of individual leaves and on the manner in which active growth proceeded. This was apparent both in total growth and in rapidity of growth. Some part of a normal plant was

always growing rapidly, and this region of active growth moved up the stem from leaf to leaf as the new leaves emerged, but in diseased plants this region of rapid growth was less apparent at all times and the rapid area increase could not be followed continuously from leaf to leaf. The mosaic plants produced smaller, and in some cases more numerous leaves, but the total area of leaf surface was consistently less in the diseased plants.

The mere fact, however, that in each case mosaic plants were provided with a smaller working leaf area would have no bearing on the efficiency of the leaves themselves per unit of area. Indeed, smaller leaves might be considered an advantage since they shade each other less and so receive a greater total amount of light per plant. In the mosaic pattern formed by healthy foliage some leaves or parts of leaves are always in the shadow, especially the larger ones towards the base. The advantage of better and more light apparently did not offset the disadvantage of subnormal chloroplasts, for the starch and sugar content in diseased plants was far below that of a normal plant on a leaf-area unit basis. Even in the slightly affected plants in which the mosaic spots were not conspicuous the chloroplasts were incapacitated to a considerable degree and did not function so well in fixing carbon (table 4). The fact that the severely diseased plants seemed to fix more carbon per unit area than the slightly diseased plants may be explained by the inaccuracy of the measurements caused by the wrinkled surface.

Functions in which chloroplasts are not involved seemed to be little affected by the disease. The ash percentage in the diseased plants varied little from that in the normal plant, indicating that roots are nearly normal despite mosaic on the above ground parts. These results seem to locate the detrimental effects of the disease in the leaves.

SUMMARY

Daily measurements were made from July 25 to September 3 of all leaflets on a normal potato plant, on plants slightly affected with mosaic, and on plants severely affected with mosaic.

The mosaic plants tended to produce more leaves than the normal plant, but the leaf surface of the former was less than that of the latter, since the smaller number of leaves on the normal plant was more than offset by the greater size of the leaves.

The mosaic plants lengthened more slowly than the normal plant, but gave the impression of greater height because of their spindling growth as contrasted with the stocky growth of the normal plant.

Green weight per unit area of leaves became smaller as the disease affected the plants more severely, but dry weight in mosaic plants was greater per unit area than in the normal plant.

Growth in mosaic leaflets paralleled that in normal leaflets, but always at a slower rate. Mosaic leaflets did not show the marked early rapid growth of normal leaflets, and soon reached the limit of increase in area, so that eventually all the leaves were fairly uniform in size. Normal leaves, on the other hand, continued to grow steadily and maintained a variety of sizes.

In both normal and mosaic plants tip leaflets grew more rapidly than side leaflets, and side leaflets nearest the tip grew more rapidly than those farther back on the leaf petiole. The growth of the

leaflets was always greater in width than in length, and their rate of growth was inversely correlated with their size, the growth rate decreasing as the area of the leaflet increased.

Chemical analysis of the same plants which had been measured for growth showed an ash content lower in normal than in mosaic plants, but this ash content, in proportion to the dry weight of the roots of normal and mosaic plants, showed only a slight reduction in the absorbing ability of the root system of plants affected by disease.

The carbon content in grams seemed to be determined by the size of the plant and varied accordingly, but the carbon percentage of the dry weight varied little in normal and diseased plants.

The special contribution of the entire work, both on growth and analysis, lies in the data which show that mosaic, either slight or severe, reduces the efficiency of a unit area of foliage exposed, as measured by the amount of carbon dioxide fixed in the body of the plant as carbon or stored in the aboveground part of the plant or in the tubers as starch. The point to be emphasized is that the carbon-fixation apparatus does not work so efficiently in mosaic-affected plants as it does in normal plants.

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ORIGIN AND PRODUCTION OF MORPHOLOGIC AND PATHOGENIC STRAINS OF THE OAT SMUT FUNGI BY MUTATION AND HYBRIDIZATION[1]

By C. S. Holton

Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture[2]

INTRODUCTION

The phenomenon of physiologic specialization in the oat-smut fungi, *Ustilago avenae* (Pers.) Jens. and *U. levis* (Kell. and Sw.) Magn., has received considerable attention during the last decade. In 1924 Reed (*5*)[3] gave the first evidence that pathogenically different entities exist within these two species when he described two physiologic races each of *U. avenae* and *U. levis*. Further evidence of specialization in the oat smut fungi was presented by Sampson (*10*), who designated these specialized entities as biological species.

Differences between forms of the oat smut fungi with regard to characteristics on artificial culture media have been demonstrated by Rodenhiser (*9*), who described 18 physiologic forms of *Ustilago avenae* and 5 of *U. levis*. In a second report Reed (*6*) presented further evidence of physiologic races in the oat smuts, and in 1929 (*7*) he described and classified 11 races of *U. avenae* and 5 of *U. levis*. More recently Reed and Stanton (*8*) reported the occurrence of a hitherto unknown specialized race of *U. levis* on Fulghum, an extensively grown red oat (*Avena byzantina* C. Koch). It seems apparent, then, that the number of recognized races of oat smut fungi is increasing and that there is considerable economic significance in this fact (*8*).

In view of the fact that these pathogenic strains[4] of *Ustilago avenae* and *U. levis* exist and that their number apparently is increasing, it would seem worth while to determine the nature of their origin.

Considerable speculation has centered about the role of hybridization and mutation in this connection. In fact, there seemed to be little reason to doubt that new strains would arise as a result of hybridization when it was reported by Hanna and Popp (*1*) and Holton (*2*) that crosses between monosporidial lines of *Ustilago avenae* and *U. levis* produced hybrid chlamydospores on the host. On the other hand, a very low degree of viability occurred in the F_1 sporidia from chlamydospores of these species hybrids (*2*), and it appeared that this would weaken the probability of the origin of new forms by this

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[2] Sincere appreciation is expressed to Dr. F. D. Heald, head of the division of plant pathology, and Dr. H. F. Clements, associate professor of botany, State College of Washington, and to A. M. Schlehuber, of the Washington Agricultural Experiment Station, for critical reading of the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 317.

[4] Physiologically distinct entities within *Ustilago avenae* and *U. levis* have been designated variously as physiologic races, biological species, and physiologic forms. These terms may be interpreted as implying one or more differences in physiologic properties. The term "pathogenic strain" is used in this discussion because only the pathogenic properties have been considered, and it seems desirable to use a term which has a definitely limited denotation.

means. As a matter of fact, after further studies, the writer (*3, p. 32*) stated that—

> in view of the high percentage of nonculturability of the hybrid sporidia the probability that this source of new physiologic forms of *Ustilago avenae* and *U. levis* will be a significant factor in the epiphytology of oat smuts seems very remote.

This statement was based on the results of studies in which crosses between monosporidial lines from chlamydospores of species hybrids were used for inoculation studies.

In view of the fact that only about 1 percent of the F_1 sporidia of the species hybrids would grow in culture (*2, 3*), the tests were necessarily rather limited in scope. The results of more recent studies, however, in which the F_1 chlamydospores were used for inoculum instead of the crosses between F_1 monosporidial lines, indicate rather conclusively that hybridization between species plays an important role in the development of new pathogenic strains of the oat smut fungi. Furthermore, by using F_1 chlamydospores for inoculum instead of crosses between F_1 monosporidial lines, a more comprehensive understanding of the origin of the buff smut of oats (*2, 3*) and its relationship to *Ustilago avenae* and *U. levis* has been brought about.

MATERIAL AND METHODS

A Gothland strain of *Ustilago avenae*, Reed's physiologic race I (*7*), a Monarch strain of *U. levis*, Reed's physiologic race I (*7*), and a pure-line strain of the buff smut fungus were used in these investigations. Specimens of Reed's (*7*) physiologic races were supplied by the late J. A. Faris, who had obtained them from Dr. Reed. The varieties Gothland (C. I.[5] 1898) and Monarch (C. I. 1896) were used as differential host testers, and Anthony (C. I. 2143) was used for the susceptible check to determine the viability of the inoculum under the conditions of the various tests.

Four monosporidial lines were isolated from a germinating chlamydospore of each of the three strains to be tested (*3*). Intraspecific and interspecific crosses were obtained by inoculating Anthony oats with sexually compatible combinations of these monosporidial lines (*3*). Inasmuch as the F_1 sporidia are so weakly viable, the F_1 chlamydospores produced by the specific crosses were used for the pathogenicity tests. The three varieties Anthony, Gothland, and Monarch were inoculated with the F_1 chlamydospores, and selections were made of *Ustilago levis* and the buff smut fungus in the F_2 on Gothland and of *U. avenae* in the F_2 on Monarch. The pathogenic reactions of these selections and the pure-line parental strains were compared on the host testers.

It should be stated here that the factors for echinulate spore walls and brownness of chlamydospores of oat smut fungi are dominant over factors for smooth spore walls and hyalineness (*3, 4*). Hence in *Ustilago avenae* × *U. levis* hybrids, smooth spore segregates are homozygous for that character. Also in *U. avenae* × buff smut hybrids, smooth hyaline chlamydospore segregates supposedly are homozygous for these characters. Therefore, *U. levis* and buff smut segregates from *U. avenae* × *U. levis* and *U. avenae* × buff smut

[5] C. I. denotes accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

hybrids, respectively, are morphologically pure lines and may be obtained as early as in the F_2 generation.

Since the factors for echinulation and brownness are dominant, the F_2 *Ustilago avenae* segregates may be either homozygous or heterozygous, and consequently three or more generations will be required for selection of a morphologically homozygous strain. Thus, in view of the writer's knowledge of the nature of the inheritance of chlamydospore characteristics, it is relatively simple to obtain morphologically homozygous strains of *U. avenae*, *U. levis*, and the buff smut fungus from hybrids between these organisms. On the other hand, very little is known regarding the nature of the inheritance of factors for pathogenicity in the oat smut fungi and it cannot be assumed that a morphologically homozygous strain selected from a hybrid between two pathogenically different strains is pathogenically homozygous. However, by determining the pathogenicity of such a strain in the F_3 generation some indication as to its pathogenic potentialities can be gained. This method was used in these studies, and any F_3 segregate with smooth spores that was obviously different from the parent lines in its pathogenic reaction was considered as a new pathogenic strain, even though it is recognized that there may be further segregation for pathogenicity in such a strain.

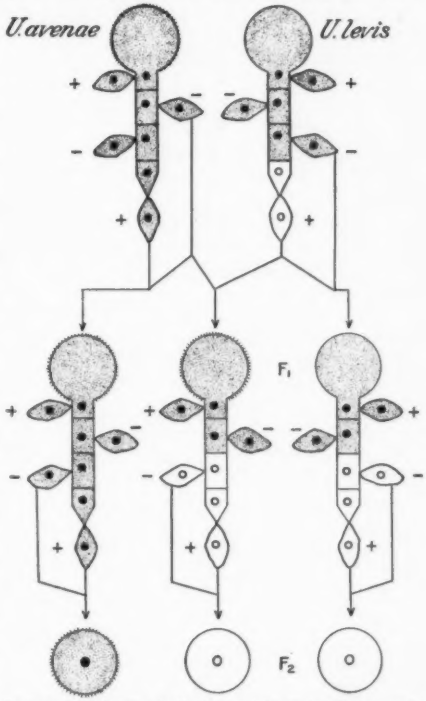

FIGURE 1.—Diagram indicating origin and subsequent appearance of the buff smut fungus which has hyaline chlamydospores. The shading indicates the cells in which the factor or factors for color are carried, and the absence of shading indicates those from which such factor or factors are absent. The nuclei are indicated by dots and circles. The plus and minus signs indicate sporidia of opposite sex.

RESULTS

MUTATION

In a previous paper on hybridization between *Ustilago avenae* and *U. levis* (*2*) a new type of oat smut was described as resulting from a cross between two monosporidial lines which were derived from a species-hybrid chlamydospore. This new smut, which is caused by a fungus that has smooth hyaline chlamydospores, has been designated as the "buff smut of oats" because of the characteristic buff color of the diseased panicles. Inasmuch as it had appeared first in the F_2 generation of a *U. avenae* × *U. levis* hybrid, it was suggested in a

later report (*3*) that perhaps the buff smut developed as a result of the loss of a factor or factors for color or that possibly it was the result of a difference in the factor or factors for color in *U. avenae* and *U. levis*. There are indications, however, that the buff smut fungus has a closer genetic relationship to *U. levis* than to *U. avenae*. Like *U. levis* it has smooth spores, and it crosses with this species more readily than with *U. avenae*. Even so, until now it had not been proved that the buff smut fungus arose directly from *U. levis* and not as a result of hybridization between *U. avenae* and *U. levis*.

The origin of the buff smut of oats has now been established with reasonable certainty and is indicated in table 1 and illustrated diagrammatically in figure 1. Monosporidial lines were obtained by isolating the four primary sporidia from the promycelium of germinating chlamydospores of *Ustilago avenae* and *U. levis*, respectively. Four selfed lines of each species and eight interspecific hybrids were produced by crossing the eight monosporidial lines in all sexually compatible combinations. The color characteristics of the chlamydospores of these selfed lines and species hybrids in the F_1 and F_2, are indicated in table 1.

TABLE 1.—*Color segregation in the chlamydospores of selfed lines and hybrids of Ustilago avenae and U. levis, showing the crosses and generation in which the buff smut appeared first*

[B=brown; SB=some brown; SH=some hyaline]

Generation	Selfed lines of—								*U. avenae*-10 × *U. levis*-11							
	U. avenae-10				*U. levis*-11											
	1×2[1]	1×3	2×4	3×4	1×2	1×3	2×4	3×4	1×2	1×3	2×1	2×4	3×1	3×4	4×2	4×3
F_1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
F_2	B	B	B	B	SB / SH	SB / SH	B	B	B	B	SB / SH	B	SB / SH	B	B	B

[1] The numbers refer to the position of the sporidia on the promycelium, beginning at the apex.

The four selfed lines of *Ustilago avenae* produced brown chlamydospores in the F_1 and F_2 generations. In the four *U. levis* selfed lines, all the F_1 chlamydospores were brown while in the F_2 generation brown and hyaline chlamydospores appeared in two of the lines. As shown in table 1, sporidium no. 1 was a component of both the selfed lines that produced the hyaline chlamydospores in the F_2 generation. In the 8 species hybrids, the F_1 chlamydospores were brown, and in 6 of these hybrids the F_2 segregates were likewise brown. In the other 2 species hybrids, however, there was segregation for brown and hyaline chlamydospores, and in both cases sporidium no. 1 of the *U. levis* parent was a component of the cross. On the basis of these results a diagram (fig. 1) was prepared to indicate the origin of the hyaline chlamydospores.

In the *Ustilago levis* parent, segregation for sex occurred in the second division of the diploid nucleus, and during this meiotic process the nucleus of the apical cell of the promycelium lost its faculty for producing color. It has already been reported (*3*, *4*), as indicated in table 1, that brown color is dominant over hyalineness. Consequently all the F_1 chlamydospores of crosses with the monosporidial

line that originated from the apical cell of the promycelium of *U. levis* were brown in color. When these F_1 chlamydospores germinated there was independent segregation for sex and color, or lack of color, and sporidia of opposite sex minus factors for brown color were produced, as indicated in figure 1, and in the F_2 generation the buff smut with hyaline chlamydospores appeared. Therefore, it seems rather probable that the factor or factors for color in the buff smut fungus originated through the process of mutation in *U. levis* and that its ultimate appearance was due to independent segregation of factors for sex and color which made possible a recombination of factors for hyalineness.

HYBRIDIZATION

The results of tests to compare the pathogenicity of pure lines, hybrids, and hybrid segregates of *Ustilago levis*, *U. avenae*, and the buff smut organisms are summarized in table 2. The *U. levis* strain produced 99 percent smut on Monarch and 0 percent on Gothland, while the *U. avenae* strain produced 0 percent smut on Monarch and 100 percent on Gothland. The buff smut strain produced 55 percent smut on Monarch and 0 percent on Gothland, indicating that it is somewhat less virulent on Monarch than the *U. levis* strain. These figures show rather definitely that the pathogenic properties of the *U. avenae* strain are distinctly different from those of the *U. levis* and buff smut strains, and for this reason these strains were considered as suitable material to use in hybridization studies.

TABLE 2.—*Percentages of smutted panicles produced on oat varieties by pure lines, hybrids, and hybrid segregates of Ustilago levis, U. avenae, and the buff smut fungus* [1]

Variety of oats	Percentage of panicles smutted by—						
	U. levis	*U. avenae*	Buff smut	*U. levis* × *U. avenae*		*U. avenae* × buff smut	
				F_2	F_2 *U. levis* segregate F_3	F_2	F_2 buff segregate F_3
Gothland	0.0	100.0	0.0	22.0	92.0	3.0	100.0
Monarch	99.0	.0	55.0	21.0	45.0	6.0	22.0

[1] The low percentages of smut produced in the F_2 of the *U. levis* × *U. avenae* and *U. avenae* × buff smut hybrids is no doubt explainable on the basis of a low degree of viability of F_2 sporidia and the production of individuals, through segregation and recombination of factors for pathogenicity, that were not pathogenic on these 2 varieties.

As indicated in table 2, the *Ustilago levis* × *U. avenae* hybrid produced 22 and 21 percent smut on Gothland and Monarch, respectively, in the F_2 generation, and on each variety both *U. avenae* and *U. levis* types were found. From the F_2 generation on Gothland a pure strain of *U. levis* was selected that produced, in the F_3 generation, 92 percent smut on the same variety and 45 percent on Monarch. These figures are in contrast to 0 and 99 percent of smut produced on Gothland and Monarch, respectively, by the *U. levis* parent and 100 and 0 percent produced on Gothland and Monarch, respectively, by the *U. avenae* parent. It is evident, therefore, that by crossing *U. avenae*

and *U. levis* a synthetic strain of *U. levis* has been produced which is more widely virulent than either parent, and that its production was brought about by segregation and recombination of factors for pathogenicity present in the parental lines.

Referring again to table 2, it can be seen that in the F_2 generation the *Ustilago avenae* × buff smut hybrid produced 3 percent of smut on Gothland and 6 percent on Monarch, segregates of both types appearing. It was notable that some of the Gothland plants were smutted with the buff smut, whereas this variety was immune from the parent strain of the buff smut. This buff smut segregate from Gothland produced, in the succeeding generation, 100 percent of smut on Gothland as compared with 0 percent of smut produced on the same variety by its buff smut parent. On Monarch the new buff smut strain produced 22 percent of smut as compared with 0 and 55 percent produced on the same variety by the *U. avenae* and buff smut parents, respectively. Thus, apparently the new buff smut strain is decidedly more virulent on Gothland but somewhat less virulent on Monarch than the parental buff smut strain. Furthermore, it is equally as virulent on Gothland and significantly more virulent on Monarch than its *U. avenae* parent. It appears, therefore, to have pathogenic properties not possessed by either parent and apparently is a new strain of the buff smut fungus that has arisen through segregation and recombination of factors for pathogenicity in a hybrid between a strain of *U. avenae* and a strain of the buff smut fungus.

SUMMARY AND CONCLUSIONS

A new type of oat smut, described and designated in previous reports (*2*, *3*) as "buff smut", has been found to be the result of mutation in *Ustilago levis*. The buff smut fungus has hyaline chlamydospores, but in all other morphological characteristics it resembles *U. levis*. Consequently, the change that occurred apparently involved only the factor that determines color in the chlamydospores. Evidence is presented which indicates rather definitely that the change in the color factor occurred during meiosis in a germinating chlamydospore, with the result that 1 of the 4 haploid nuclei lost the factor for brownness. This nucleus became located in the apical cell of the promycelium, and all crosses with the monosporidial line derived from this cell gave rise to heterozygous brown chlamydospores in the F_1 generation and the hyaline buff smut spores were segregated in the F_2. Because factors for brown are dominant over factors for hyalineness, all the F_1 chlamydospores were brown, and the appearance of the hyaline chlamydospores in the F_2 is explained on the basis of independent segregation of factors for sex and color of chlamydospores.

A new pathogenic strain of *Ustilago levis* that attacks Gothland and Monarch was produced by crossing a Gothland strain of *U. avenae* with a Monarch strain of *U. levis*. The synthetic strain of *U. levis* was selected in the F_2 generation from Gothland, and it infected both varieties in the F_3, being almost as virulent on Gothland as the *U. avenae* parent but somewhat less virulent on Monarch than the *U. levis* parent. Also, a new pathogenic strain of the buff smut fungus that attacks Gothland and Monarch was produced by crossing the Gothland strain of *U. avenae* with the Monarch buff smut strain which would not attack Gothland. The synthetic strain of the buff smut

fungus was selected from Gothland in the F_2 generation, and in the F_3 it was equally as virulent on Gothland as the *U. avenae* parent but somewhat less virulent on Monarch than the buff smut parent.

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